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(54) Title: USE OF SYNTHETIC PEPTIDES TO INDUCE TOLERANCE TO PATHOGENIC T AND B CELL EPITOPES OF AUTOANTIGENS

(57) Abstract

The present invention relates, in general, to the use of synthetic peptides to induce tolerance to immunogenic peptides. In particular, the present invention relates to a method of inducing tolerance in a mammal to an immunogenic peptide or protein comprising administering to a mammal a synthetic toleragen comprising a hydrophobic peptide linked to the N-terminus or C-terminus of the immunogenic peptide or protein, under conditions such that the tolerance is induced.

USE OF SYNTHETIC PEPTIDES TO INDUCE TOLERANCE TO PATHOGENIC T AND B CELL EPITOPES OF AUTOANTIGENS

BACKGROUND OF THE INVENTION

5   This is a continuation-in-part of Application Number 07/833,429, filed February 10, 1992, which is a continuation-in-part of Application No. 07/591,109, filed October 1, 1990, which is a continuation-in-part of Application
10   Number 93,854, filed September 8, 1987, now U.S. Patent 5,019,387, the entire contents of which are hereby incorporated by reference.

Field of the Invention

      The present invention relates, in
15   general, to the use of synthetic peptides to induce tolerance to immunogenic peptides. In particular, the present invention relates to a method of inducing tolerance in a mammal to an immunogenic peptide or protein comprising
20   administering to a mammal a synthetic toleragen comprising a 2 to 20 amino acid hydrophobic peptide linked to the N-terminus or C-terminus of the immunogenic peptide or protein, under conditions such that the tolerance is induced.

25   ### Background Information

      Many autoimmune diseases in animals and man are characterized by T and B cell responses to pathogenic epitopes on self antigens (Immunotherapy of Diabetes and Selected Autoimmune
30   Diseases, G.S. Eisenbarth (ED) CRC Press, Boca Raton, 1989; Current Therapy in Allergy,

Immunology, and Rheumatology-3. L.M.
Lichtenstein, et al, B.C. Decker, Inc., Toronto,
1988). Examples of autoimmune diseases or disease
models that are caused by autoreactive B cells

5   responses are listed in Table 1. Examples of
autoimmune diseases or disease models that are
caused by autoreactive T cell responses are listed
in Table 2. A method to tolerize human
lymphocytes to not respond to pathogenic T and B

10   cell epitopes of autoantigens that otherwise
induce immune responses that cause tissue damage
would represent a significant advance in the
therapy of autoimmune diseases. Similarly,
multiple clinical situations exist outside the

15   setting of autoimmune disease, in which B and T
cell responses are harmful and would
advantageously be shut off or decreased. Examples
of pathogenic non-autoimmune antibody responses
are antibody responses to ABO incompatible

20   erythrocytes. A method to induce tolerance
against this type of immunogen would be a powerful
tool for treatment of a number of similar
conditions.

           Recently, it has also become clear that
25   tissue destruction in certain infectious diseases
is caused by immune responses against normal
tissue that are induced by infectious agents. For
example, in HTLV-I infection, the clinical
syndrome of HTLV-1 associated myelopathy (HAM) has

30   been shown to be associated with the induction of
cytotoxic T lymphocytes reactive with a specific
region (SP4A1) OF HTLV-1 gp46 envelope
glycoprotein (S. Jacobson, et al, _J. Immunol._.
146:1155-1162, 1991). Similarly, lymphocytic

35   pneumonitis in HIV infection has been shown to be
associated with the presence in lung lymphocytes
of CTL specific for HIV infected cells (_AIDS_,

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B.D. Walk r, et al, 4:177, 1990). In both HIV and
HTLV-1 infection, it is thought that certain
manifestations of the disease are caused by the
induction of anti-viral immune responses that
5   cross-react with normal human host antigens
(G.W. Hoffman, et al, Proc. Natl. Acad. Sci. USA
88:3060-3064, 1991; H. Wigzell, et al, FASEB J.
p.2406-2410, 1991; H. Golding, et al, J. Clin.
Invest. 83:1430-1435).
10   Robinson et al have demonstrated that
antibody responses to HIV envelope gp41 epitopes
enhance HIV infectivity (W.E. Robinson, et al,
Proc. Natl. Acad. Sci. USA, 86:4710, 1989).
Recently, evidence has been presented that many if
15   not all of the manifestations of AIDS may be
caused by an autoimmune response to HLA antigens
that are induced by HIV viral proteins that share
sequence homologies with normal host HLA molecules
(G.W. Hoffman, et al, Prac. Natl. Acad. Sci. USA
20   88:3060-3064, 1991; H. Wigzell, et al, FASEB J.
p.2406-2410, 1991; H. Golding, et al, J. Clin.
Invest. 83:1430-1435). Thus, a method of
induction of tolerance (non-responsiveness) to
pathogenic HIV or HTLV-1 protein epitopes (or to
25   epitopes of any other infectious agent that
induces autoreactive immune responses), would be
an important and novel mode of preventing
infectious tissue damage.
          The ability to induce tolerance to an
30   immune response induced by an infectious agent to
prevent tissue destruction has been proposed as a
method of treatment of Herpes simplex virus
(HSV-1) corneal inflammation (R.L. Hendricks, et
al, J. Immunol. 142:263-269, 1989).
35   The form of antigen has been suggested
to be important regarding determination of whether
a protein antigen is an immunogen or a toleragen

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(Reviewed in Weigle (1989) The role of the
physical state of human gamma globulin in the in
vivo and in vitro induction of immunological
tolerance. Chapter 5G, Vol. II, p 51-57). Whereas

5   high molecular weight aggregated gamma globulin is
a potent immunogen, low molecular weight globulin
is a toleragen (W.O. Weigle, Chpt. 5G, Vol. II,
p.51-57, 1989). In this case, the ability of
aggregated gamma globulin to induce endogenous IL1

10 has been suggested as the mode of immunogenicity
of aggregated gamma globulin (L.C. Gahring, et al,
J. Immunol. 145:1318-1323, 1990)
        Others have suggested that some T cell
epitopes are inherently immunogenic and some are

15 toleragenic (D.R. Milich, et al, J. Immunol.
143:3148-3156, 1989). Milich has converted
toleragenic epitopes of Hepatitis B core antigen
to immunogenic epitopes by single amino acid
substitutions in the T cell epitopes (D.R. Milich,

20 et al, J. Immunol. 143:3148-3156, 1989).
Benacerraf has suggested that freely diffusible
antigens are toleragens whereas particulate
antigens that are concentrated in cells of the
reticuloendothelial system are immunogenic

25 (Benacerraf, B. Properties of antigens in relation
to responsiveness and non-responsiveness, in
Immunological Tolerance, M. Landy, W. Braun, Eds.
Academic Press, NY, NY 1969). In contrast, Nossal
reported that the particulate polymeric antigen

30 flagellin was a potent toleragen, and induced
tolerance to antibody responses to the Salmonella
flagella when injected into neonatal rats (Nossal,
G Antigen Dosage in Relation to Responsiveness and
Non-responsiveness, in Immunological Tolerance, M.

35 Landy, W. Braun, Eds. Academic Press, NY, NY
1969). Finally, immunogenicity versus
toleragenicity of antigens has been proposed to be

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due to their affinity of binding to MHC and TCR
molecules (rev. in Spent et al, Science 248:1357-
2363, 1990).

5   The present invention provides a method
of modification of peptide immunogens whereby the
modification changes a potent immunogen into a
potent toleragen. The invention is based on the
unexpected observation that the F-domain of HIV-1
gp41 confers to an antigen the ability to be a
10   toleragen. Specifically, the hydrophobic N-
terminal 12 amino acids of the gp41 envelope
protein that mediate fusion of HIV to uninfected
cells, the fusogenic (F) domain (M.L. Bosch, et
al, Science, 244:694-697, 1989), were added C-
15   terminal to the highly immunogenic T1-SP10 and T1-
SP10(A) peptides (Table 3) (T.J. Palker, et al, J.
Immunol. 142:3612-3619; M.K. Hart, et al, J.
Immunol. 145:2677-2685, 1990; M.K. Hart, et al,
Proc. Natl. Acad. Sci. USA 88:9448-9452, 1991).
20   When used as an immunogen in chimpanzees, the T1-
SP10IIIB and the T1-SP10IIIB(A) peptides were
potent immunogens, whereas the F-T1-SP10IIIB(A)
peptide (M.K. Hart, et al, Proc. Natl. Acad. Sci.
USA 88:9448-9452, 1991) were not as immunogenic at
25   either low (.1mg/kg) or at high (.5 mg/kg) doses.
Moreover, challenge of the animals with the highly
immunogenic T1-SP10IIIB(A) peptide at month 16 of
the immunization schedule proved that the F-T1-
SP10II_B(A) immunized animals were tolerant to the
30   T1-SP10IIIB(A) HIV gp120 env determinants.

                    SUMMARY OF THE INVENTION

          It is a general object of this invention
to provide a method of inducing tolerance in a
mammal to an immunogenic peptide or protein.

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It is a specific object of this
invention to provide a method of inducing
tolerance in a mammal to an immunogenic peptide or
protein comprising administering to the mammal a
5   synthetic toleragen comprising a hydrophobic
peptide linked to the N-terminus or C-terminus of
the immunogenic peptide or protein, under
conditions such that the tolerance is induced.
      Further objects and advantages of the
10 present invention will be clear from the
description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

      Figure 1. Antibody Titers in ELISA
Assay Against Immunizing Peptide Over Time In
15 Chimpanzees Immunized with HIV Env Synthetic
Peptides.
      Figure 2. Peripheral Blood Mononuclear
Cell Proliferative Responses to the T1-SP10IIIB(A)
Peptide in 7 Day Tritiated Thymidine Incorporation
20 Assays.
      Figure 3. PBMC Proliferative Responses
of Chimpanzees Immunized with T1-SP10 Peptides and
F-T1-SP10 Peptides to PHA.
      Figure 4. Elution Profile of SP10MN(A)
25 Over a G-75 Sephadex Column.
      Figure 5. Elution of T1-SP10MN(A) Over
a G-75 Sephadex Column.
      Figure 6. Elution of F-T1-SP10MN(A)
Over a Sephadex G-75 Column.
30 Figure 7. Elution of F-SP10MN(A) Over
G-75 Column.
      Figure 8. Results of DSP Cross-linking
Analysis Using F-T1-SP10IIIB(A) Peptide.
      Figure 9. Hypothetical Model of F-T1-
35 SP10IIIB(A) in Aqueous Solution.

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Figure 10. Variants of T1-SP10 peptides
derived from HIV MN and IIIB Envelope Sequences.

Figure 11. Time course of PBMC 3H-
thymidine incorporation responses to HIV Th-B
5   peptide, T1-SP10IIIB(A), in chimpanzees immunized
with HIV envelope synthetic peptides. Animals 884
(Figure 1A) and 1028 (Figure 1B) received the Th-B
peptide, T1-SP10IIIB, initially (months 1-5), then
the Th-B peptide, T-SP10IIIB(A) (month 6-8).
10   After a boost with the Th-B peptide T-SP10IIIB(A)
at month 14, both animals 884 and 1028 were
immunized with the HIVMN Th-B peptide, T-
SP10MN(A). Panels C and D show the responses of
animal 1045 (Panel C) and 1070 (Panel D) to the
15   HIVIIIB F-Th-B peptide (month 1-14), HIVIIIB Th-B
peptide (month 16) and HIVMN Th-B peptide (months
17-19). All immunizations were with the indicated
peptide in IFA, except all immunizations for
animal 1028 after month 4, which were with
20   peptides in PBS alone. Solid lines show data for
peak proliferative reponses (Δcpm) to a wide dose
range of HIVIIIB Th-B peptide. Dotted lines
indicate peak proliferative response (Δcpm) to a
wide dose range of the HIVMN Th-B peptide.
25   Figure 12. Time course of PBMC 3H-
thymidine incorporation response to PHA in
chimpanzees immunized with HIV envelope synthetic
peptides. Immunizations and chimpanzees as in
Figure 11.
30   Figure 13. Time course of PBMC 3H-
thymidine incorporation response to candida
antigen in chimpanzees immunized with HIV envelope
synthetic peptides. Immunizations and chimpanzees
as in Figure 11.
35   Figure 14. Time course of absolute
numbers of lymphocytes and lymphocytic subsets in
chimpanzees immunized with HIV envelope synthetic

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peptides.   Immunizations and chimpanzees as in
Figure 11.   Points represent cell number/mm3 of
peripheral blood lymphocytes and lymphocyte
subsets.   The elevated cell numbers in animal 1028
5   at month 4 coincided with an abscess at the
injection sites.
          Figure 15.   HIV envelope hybrid
synthetic peptides induced anti-HIV neutralizing
antibodies in goats.   Goat 102A was immunized with
10   3 mg of the F-Th-B peptide, F-T1-SP10IIIB(A) and
goat 104A was immunized with the HIVIIIB Th-B
peptide, T1-SP10IIIB.   Immunizations were in CFA
(first dose) and IFA (doses 2-4).   Neutralizing
titers are titers at which reverse transcriptase
15   production was inhibited by 90% or greater.

          DETAILED DESCRIPTION OF THE INVENTION

          The present invention relates to a
procedure whereby protein immunogens are
derivatized by either synthesizing a hydrophobic
20   amino acid sequence of 2 to 20 amino acids in
length, N-terminal to the immunogenic protein or
protein fragment, or covalently linking a
hydrophobic peptide fragment of 2 to 20 amino
acids in length N-terminal to the immunogenic
25   protein or protein fragment, to yield an immunogen
capable of inducing tolerance to the protein
immunogen when administered to a mammal such as a
primate (more preferably, humans).
          In a preferred embodiment, the
30   hydrophobic peptide is 5 to 15 amino acids in
length.   In yet another preferred embodiment, the
hydrophobic peptide is 7 to 13 amino acids in
length.   In a further embodiment, the length of
the hydrophobic peptide is 7, 8, 9, 10, 11, 12, or
35   13 amino acids in length.   In yet another

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emb diment, the hydrophobic peptide is at least 5
amino acids in length (more preferably, at least
10 amino acids in length).

5
        Alternatively, immunogenic proteins
known to be the targets of autoantibody or auto-T
cell responses can be constructed using
recombinant DNA technology to form new toleragens
containing hydrophobic N-terminal regions as
described above. While an advantageous
10   construction of the invention is for the
hydrophobic sequence to be N-terminal to the B or
T cell epitope, in certain circumstances it may be
advantageous to have the hydrophobic sequence C-
terminal to the B or T cell epitope.

15   The hydrophobic region can be a fusion
protein from HIV or HIV-related viruses (see
Table 5), or can be a hydrophobic sequence of
amino acids that is either randomly selected or is
from a non-HIV related protein.

20   An example of this invention for
inducing tolerance to antibodies against
autoantigens is for the treatment of myasthenia
gravis, whereby the F-sequence is synthesized N-
terminal to the main immunogenic region of the
25   acetylcholine receptor, **WNPADYGGIK** or **WNPDDYGGVK**
(I. Papdouli, et al, Biochem. J. 269:239-245,
1990). The resulting immunogen is
AVGIGALFLGFLWNPADYGGIK or AVGIGALFLGFLWNPDDYGGVK.

        Another example of a B cell toleragen is
30   a hybrid protein comprising the HIV fusion domain
synthesized either linearly N-terminal to B cell
peptide epitopes of the insulin molecule or
covalently linked to the whole insulin molecule or
covalently linked or constructed using recombinant
35   DNA techniques to a peptide insulin fragment or to
the whole insulin molecule. The resulting
immunogen is AVGIGALFLGFL-insulin or AVGIGALFLGFL-

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insulin peptide fragment. These types of
toler**a**gens can be used to prevent the onset of
juvenile diabetes mellitus, (J.P. Palmer, et al,
Science 222:1337-1339, 1983; B.M. Dean, et al,
5   Diabetologia 23:339-342, 1986) and to treat
patients with insulin antibodies in the setting of
insulin resulin resistance (J.D. Schnatz,
Dolovich, et al, J. Allergy, 46:127-1137, 1970).
Another example of a B cell toleragen is
10   a hybrid protein comprising the HIV fusion domain
synthesized either linearly N-terminal to B cell
peptide epitopes of the TSH receptor molecule or
covalently linked to the whole TSH receptor
molecule or covalently linked or constructed using
15   recombinant DNA techniques to a peptide TSH
receptor fragment or to the whole TSH receptor
molecule. The resulting immunogen is
AVGIGALFLGFL-TSH receptor or AVGIGALFLGFL-TSH
receptor peptide fragment. These types of
20   toleragens can be used to treat autoimmune thyroid
disease (Graves' Disease) (T. Mori, et al,
Biochem. & Biophy. Res. Comm. 178:165-172, 1991;
M. Murakami, et al, Biochem. & Biophy. Res. Comm.
171:512-518, 1990). Table 6 summarizes B cell
25   epitopes on the thyrotropin (TSH) receptor to
which Graves' patient sera bind (T. Mori, et al,
Biochem. & Biophy. Res. Comm. 178:165-172, 1991;
M. Murakami, et al, Biochem. & Biophy. Res. Comm.
171:512-518, 1990; O. Takai, et al, Biochem. &
30   Biophy. Res. Comm. 179:319-326, 1991;
T. Piraphatdis, et al, Biochem. & Biophy. Res.
Comm. 172:529-536, 1990). Of interest is the
sequence YYVFFEEQEDEIIGF identified by 2 studies
that inhibits the TSH activity of the
35   autoantibodies (T. Mori, et al, Biochem. & Biophy.
Res. Comm. 178:165-172, 1991; O. Takai, et al,
Biochem. & Biophy. Res. Comm. 179:319-326, 1991).

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Thus constructs for inducing tolerance to anti-TSH
antibodies in Graves' disease are
AVGIGALFLGFLYVFFEEQEDEI or
AVGIGALFLGFLHQEEDFRVTCKDIQRIPSLPPSTQT or
5   AVGIGALFLGFLLRQRKSVNALNSPLHQEYEENLGDSIVGY or
AVGIGALFLGFLYYVFFEEQEDEIIGF or
AVGIGALFLGFLYKELPLLKFL.

An example of the use of this invention
in the induction of tolerance to autoimmune T cell
10   antigens is a hybrid protein comprised of the HIV
fusion domain synthesized either linearly N-
terminal to T cell peptide epitopes of the myelin
basic protein molecule or covalently linked or
constructed using recombinant DNA techniques to a
15   myelin protein molecule. The resulting immunogen
is AVGIGALFLGFL-myelin basic protein or
AVGIGALFLGFL-myelin basic protein peptide
fragment. In the case of the myelin basic protein
peptide fragment, the encephalitogenic T cell
20   epitopes are known, one of which is contained in
sequence 69-89 of bovine myelin basic protein (H.
Offner, et al, J. Immunol. 141:3828-3832, 1988).
In this case, one formulation of the toleragen is
AVGIGALFLGFLGSLPQKSQRSQDENPVVHF. These types of
25   toleragens can be used to treat experimental
autoimmune encephalomyelitis, which is thought to
be an excellent model of human multiple sclerosis
(K.W. Wucherpfenning, et al, Immunol. Today,
12:277-281, 1991). When the specific epitopes are
30   identified that are the T cell targets in multiple
sclerosis, then those sequences can be substituted
in the peptide above, and used to tolerize T cells
to the pathogenic T cell epitope of whatever the
protein antigen turns out to be involved in
35   multiple sclerosis.

Another example of this invention for
induction of tolerance to autoimmune T cell

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antigens is a hybrid protein comprising the HIV
fusion domain synthesized either linearly
N-terminal to T cell peptide epitopes of the
retinal S protein molecule or covalently linked to
the whole retinal S protein molecule or covalently
linked or constructed using recombinant DNA
techniques to retinal S antigen fragment or to the
whole retinal S antigen molecule. The resulting
immunogen is AVGIGALFLGFL-**retinal S protein** or
AVGIGALFLGFL-**retinal S protein peptide fragment**.
In the case of the retinal S protein peptide
fragment, the pathogenic T cell epitopes are
known, one of which is present in the sequence
1169-1191 of retinal S protein (H. Sanui, et al,
Exp. Med., 169:1947-1960, 1989). In this case,
one formulation of the toleragen is
AVGIGALFLGFL**PTARSVGAADGSSWEGVGVV**. These types of
toleragens can be used to treat experimental
autoimmune retinouveitis, which is thought to be
an excellent model of human inflammatory eye
diseases such as Bechet's syndrome and idiopathic
retinouveitis (H. Sanui, et al, Exp. Med.,
169:1947-1960, 1989). When the specific epitopes
are discovered that are the T cell targets in
human inflammatory eye disease, then those
sequences can be substituted in the peptide above,
and used to tolerize T cells to the pathogenic T
cell epitope of whatever the protein antigen turns
out to be in human retinouveitis.

For the treatment of pathogenic immune
responses induced by an infectious agent, an
example of the invention is the treatment of
HTLV-I associated myelopathy syndrome seen in
tropical spastic paraparesis (rev. in Jacobson et
al J. Immunol. 146:1155-1162, 1991). In this
disease, there is strong evidence that the
neurologic disease is caused by the induction of

cytotoxic T cells (CTL) against HTLV-I infected
c lls in the central nervous system (S. Jacobson,
et al, J. Immunol. 146:1155-1162, 1991).
Jacobson, et al have shown that one primary region
5   of HTLV-I env gp46 that induces CTL in tropical
spastic paraparesis (TSP) is aa196-209 of gp46 as
defined by peptide SP4a1 (S. Jacobson, et al, J.
Immunol. 146:1155-1162, 1991; T.J. Palker, et al,
J. Immunol., 142:971-978, 1989; A. Kurata, et al,
10   J. Immunol., 143:2024-2030, 1989). Thus, to treat
TSP, the present invention can be embodied by the
hybrid peptide AVGIGALFLGFLLDHILEPSIPWKSKK. When
new pathogenic CTL epitopes of HTLV-I are
discovered, the therapeutic construct can be F-X
15   where F is the hydrophobic sequence and X is the
CTL epitope of the infectious agent.
        The clinical manifestations of HIV have
been postulated to be due to autoimmune responses
induced by components of HIV that have sequence
20   homology to human MHC Class I or Class II
molecules (G.W. Hoffman, et al, Prac. Natl. Acad.
Sci. USA 88:3060-3064, 1991; H. Wigzell, et al,
FASEB J. p.2406-2410, 1991; H. Golding, et al,
J. Clin. Invest. 83:1430-1435; F. Grassi, et al,
25   J. Ex. Med., 174:53-62, 1991; J.A.T. Young,
Nature, 333:215, 1988; H. Golding, et al, J. Exp.
Med., 167:914-923, 1988). For the treatment of
HIV infection, the present invention can comprise
a series of hybrid peptides, each peptide
30   containing an N-terminal hydrophobic peptide such
as the HIV gp41 fusion domain (Table 5) and a C-
terminal peptide from each of the regions of HIV
env proteins bearing sequence homology MHC class I
or class II molecules (G.W. Hoffman, et al, Prac.
35   Natl. Acad. Sci. USA 88:3060-3064, 1991; H.
Wigzell, et al, FASEB J. p.2406-2410, 1991; H.
Golding, et al, J. Clin. Invest. 83:1430-1435; F.

Grassi, et al, <u>J. Ex. Med.</u>, 174:53-62, 1991;
J.A.T. Young, <u>Nature</u>, 333:215, 1988; H. Golding,
et al, <u>J. Exp. Med.</u>, 167:914-923, 1988) (Table 7).
Alternatively, it may be advantageous to treat HIV
5   infected individuals with F-X peptides where F is
a hydrophobic peptide such as the fusogenic domain
of HIV and X is a peptide fragment of HIV that is
immunogenic to T or B cells. In this situation, a
mixture of peptides would be used to inhibit
10   destructive anti-HIV immune responses that were
damaging host HIV-infected antigen-presenting
cells. Examples of this type of peptide are shown
in Table 3 and Figure 10, and were the peptides
used that tolerized chimpanzees in Figures 1 and 2
15   to both T1-SP10(A) determinants and to whole gp120
protein (Table 4).
         The present invention is described in
further detail in the following non-limiting
Example.

20   Example 1

         Antibody titers in ELISA assay against
immunizing peptide over time in chimpanzees
immunized with HIV env synthetic peptides are
shown in Figure 1. For animals 884 and 1028, the
25   peptide used in the ELISA assay was T1-SP10IIIB.
For animals 1045 and 1070, the peptide used in the
ELISA assay was F-T1-SP10IIIB(A). All
immunizations were in incomplete Freund's Adjuvant
(IFA) + PBS (1:1) except for animal 1028 that
30   developed IM abscesses after immunization no. 3,
and had one immunization held, then had all
subsequent immunizations in PBS only. As can be
seen, T1-SP10 peptides were excellent immunogens
in animals 884 and 1028, while T1-SP10 peptides
35   with the HIV gp41 fusion (F) domain synthesized N-

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terminal to the T1-SP10 peptide did not induce
antibody titers as high or as of long duration as
did peptides without the F domain.

It is important to note that animals
1045 and 1070 were challenged at month 16 with the
immunogen T1-SP10IIIB(A) that induced such good
antibody titers in animals 884 and 1028. Animals
1045 and 1028 did not respond to T1-SP10IIIB(A) in
IFA, thus demonstrating that they were tolerant to
the T1-SP10(A) from their prior immunizations with
F-T1-SP10IIIB(A) peptide. It is also important to
note that while boost of 884 at week 14 gave a
rise in titer to T1-SP10IIIB(A) peptide, boost of
1028 at the same time did not. Boost of 884 was
with IFA, while boost of 1028 was with no
adjuvant, but rather only PBS.

Peripheral blood mononuclear cell
proliferative responses to the T1-SP10IIIB(A)
peptide in 7 day tritiated thymidine incorporation
assays is shown in Figure 2. T1-SP10IIIB and T1-
SP10IIIB(A) peptides induced high levels of
proliferation of circulating PBMC in animals 884
and 1028. These levels fell to non-detectable
levels after a 6 month rest (month 14) but rose
again in animals 884 and 1028. Proliferative
responses in animal 1028 rose with each boost
after the 6 month rest even though the
immunizations were in PBS alone with no adjuvant.
As with B cell response, animals 1045 and 1070,
immunized with F-T1-SP10IIIB(A) peptide, did not
proliferate to T1-SP10IIIB(A) peptide. When these
latter two animals were immunized with the T1-
SP10IIIB(A) peptide that was a good immunogen in
884 and 1028, neither of the animals 1045, 1070
developed a proliferative response to T1-
SP10IIIB(A)--thus proving that the addition of the
F-domain N-terminal to the T1-SP10 peptide created

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a toleragen that tolerized animals 1045 and 1070
to the T1 and SP10 regions of gp120. As shown in
Table 4, while animals 884 and 1028 both responded
in proliferative assays to native gp120, animals
5   1045 and 1070 were tolerant to native gp120 as
well as to immunizing peptides.

PBMC proliferative responses of
chimpanzees immunized with T1-SP10 peptides and F-
T1-SP10 peptides to PHA are shown in Figure 3.
10 Data show that while animals 1045 and 1070 were
tolerant to T1 and SP10 regions of HIV gp120, PBMC
PHA responses in these animals throughout the
immunization period were normal.

Similar results were obtained with
15 peripheral blood mononuclear cell (PBMC) responses
to candida antigen in 7 day _in vitro_ stimulation
assay (not shown). Thus, while specifically
tolerant to T1 AND SP10(A), HIV env determinants,
animal 1029 and 1045 were not generally
20 immunosuppressed and could respond to candida to
PHA stimulation _in vitro_.

The effect on peptide quartenary
structure of placement of a hydrophobic sequence
N-terminal to a T cell and/or a B cell determinant
25 was examined. Using G-75 chromatography in
aqueous buffers and crosslinking of peptide
monomers using the heterobifunctional agent
Dithiobis (succinimidylpropionate) (DSP), it was
determined that addition of a hydrophobic sequence
30 such as the fusion (F) domain of HIV or HIV-like
retroviruses confers on the T1-SP10(A) or the
SP10(A) peptide the ability to form high molecular
weight aggregates, that are likely in the form of
protein micelles.
35 An elution profile of SP10MN(A) over a
G-75 Sephadex column is shown in Figure 4. 4mg of
each peptide in 2ml 50mM Tris-HCl (pH 7.5)

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c ntaining 100mM KCl and 5% glycerol, was applied
dir ctly to a 90 x 1.6 cm column of Sephadex G-75
equilibrated with 50mM Tris-HCl (pH 7.5)
containing 100mM KCl. The sizing column was
calibrated with blue dextran (200,000), bovine
serum albumin (66,000), bovine erythrocyte
carbonic anhydrase (29,000), horse heart
cytochrome C (12,400) and bovine lung aprotinin
(6,500). The elution position of each peptide was
determined by continuous measurement of eluent
absorbance at OD 280. The corresponding molecular
weight of each peptide peak was calculated from
the calibration curve of the column. Each peptide
was also applied to the column equilibrated with
the same buffer containing 0.1% C12E9
[polyoxyethylene (9) lauryl ether]. The SP10MN(A)
peptide (predicted M_r=2878) migrated as forms of
65 Da or lower. Similarly, the T1-SP10MN(A)
(predicted M_r=4771) peptide also migrated as low
mw forms ranging from 12,000 Da to 6,500 Da
(Figure 5). In contrast, both the F-SP10MN(A)
(M_r=4038) and the F-T1-SP10MN(A) peptides
(M_r=5930) contained high molecular weight forms
that migrated at ~66,000 Da (Figures 6 and 7).
Methods used in Figures 5, 6, and 7 were as in
Figure 4.

        The results of DSP cross-linking
analysis using F-T1-SP10IIIB(A) peptide are shown
in Figure 8. Lane C shows the form of the peptide
with no DSP added in PBS when run under non-
reducing conditions in SDS-PAGE. Lanes D,E,F, and
G show the effect on peptide MW when the peptide
is cross-linked with 6.25μg (D), 12.5μg (E), 25μg
(F) and 50μg (G) of DSP prior to SDS-PAGE. Lane H
shows the results of addition of 2-ME to peptide
cross-linked with 50μg of DSP and then run under
reducing conditions in SDS-PAGE showing all of the

cross-linked forms seen in lane H and all the
multiple forms seen in non-reduced, non-cross-
linked peptide seen in lane C, were now reduced to
two bands at 7000 kDa. At present, the nature of
5   the two bands in this peptide under reducing
conditions is unknown; these two bands can be
purified by cutting the bands out of preparative
gels and can be analyzed by mass spectroscopy and
sequenced. Lanes A and B show the results of
10   crosslinking F-T1-SP10IIIB(A) peptide in the
presence to Triton-X 100 1% and run under reducing
(A) and non-reducing conditions (B). Data
demonstrate that the apparent hydrophobic
interactions holding the high MW complexes
15   together are resistant to disruption by this
detergent.

A hypothetical model of F-T1-SP10IIIB(A)
in aqueous solution is shown in Figure 9. The
model shows protein micelle formation with the
20   hydrophobic fusion domain (F) regions of the
peptide in the core of the micelle with the
hydrophilic V3 regions projecting outward.

Examples 2-8

The following experimental details and
25   protocols are referenced in Examples 2-8.
Peptides: Peptides used in Examples 2-8
that follow are listed in Table 8. Peptide
synthesis was performed using either t-boc or f-
moc chemistry with a peptide synthesizer (A431;
30   Applied Biosystems, Inc. Foster City, CA).
Peptides were purified using HPLC, and the
molecular weight was determined by fast atom
bombardment mass spectrometry (R. B. Van Breeman,
North Carolina State University, Raleigh, NC)
35   using a double-focusing mass spectrometer

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(HXIIOHF; Joel Ltd., Tokyo, Japan). For Th-B and
F-Th-B peptides (Table 8), expected molecular mass
of F-Th-B peptide, F-T1-SP10IIIB(A), was 5908,
observed was 5907; expected molecular weight of
5   Th-B peptide, T1-SP10III.B, was 4061, observed was
4062; expected and observed molecular weight of
Th-B peptide, T1-SP10IIIB(a) was 4,749, and
expected and observed molecular weight of Th-B
peptide, T1-SP10MN(A), was 4771. For the peptides
10   used in the following Examples (Table 8), the
peptide amounts are gross weights. The % water by
Karl Fisher Test (Galbraith Laboratories, Inc.
Knoxville, TN) for each peptide was
F-T1-SP10IIIB(A), 6%; T1-SP10IIIB(A), 8%;
15   T1-SP10IIIB, 6% and T1-SP10MN(A), 8%.

          Animals: Chimpanzees were housed at the
New Mexico State University Primate Facility at
Alamogordo, NM. Chimpanzee No. 884 (15 yrs. old)
and 1028 (12 yrs. old) had the same sire; animal
20   1045 (10 yrs. old) and 1070 (11 yrs. old) were
unrelated to each other and to animals 884 and
1028. Outbred goats were housed at the Duke
University Animal Facilities.

          Immunizations: For goats, 3 mg of
25   peptide were injected intramuscularly in each
gluteal region in complete Freund's adjuvant (CFA)
(1st dose), then incomplete Freund's adjuvant
(IFA) (subsequent doses). For immunization of
chimpanzees, varying doses of peptides were
30   injected IM in IFA in a total volume of 4 cc, with
1 cc injected into right and left upper arms and
thighs.

          ELISA Assays: 2 μg of Th-B peptide,
T1-SP10IIIB, or rgp120IIIB (Repligen Corp.,
35   Cambridge, MD) in CBC buffer (15 mM Na_2CO_3, 35 mM
$NaHCO_3$, pH9.6) was incubated overnight in each well
of a 96 well flat bottom plate (Costar 3590).

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Wells were blocked with CBC buffer supplemented
with 3% bovine serum albumin (BSA) for at least 2
hrs and then were washed 3 times with PBS, 0.05%
Tween 20. Primary antibody at various

5   concentrations in serum diluent (95 ml PBS, 0.05%
Tween 20, supplemented with 5 g BSA in 2 ml normal
serum from same species as secondary antibody) was
incubated for 90 min at 20°C. After washing three
times, alkaline phosphatase-conjugated secondary

10   antibody was added to each well (60 min at RT) and
the plates washed. Substrate (1 mg/ml
p-nitrophenyl phosphate, Sigma Chemical Co.,
St. Louis, MO) in 0.05M CBC-0.002M $MgCl_2$ was added
to each well, and plates developed (60 min, 20°C)

15   in the dark and read at 405 nm on an ELISA reader
(Anthros; Denley Instruments Co., Durham, NC).
Endpoint ELISA antibody titers were defined as the
serum titer at which the experimental/control
(E/C) OD value $\geq$ 3.0.

20   HIV Neutralization Assays: The ability
of chimpanzee or goat serum antibodies to
neutralize HIV was determined in syncytium
inhibition assay and reverse transcriptase
inhibition assay as previously described (Palker

25   et al, J. Immunol. 142:3612 (1989); Palker et al,
Proc. Natl. Acad. Sci. USA 85:1932 (1988)). Sera
were heat inactivated (30 min, 56°C) prior to each
assay.

    PBMC Isolation and In Vitro ^{3}H-Thymidine

30   Incorporation Assays: Chimpanzee or goat PBMC was
isolated by standard density centrifugation
techniques (Palker et al, J. Immunol. 142:3612
(1989); Haynes et al, Science 215:298 (1982)). In
vitro assays of ^{3}H-thymidine incorporation were

35   performed as described (Palker et al, J. Immunol.
142:3612 (1989); Hart et al, J. Immunol. 145:2697
(1990)). For chimpanzee PBMC assays, in vitro

cultures were performed using 10% normal
chimpanzee serum. Antigens used in PBMC
proliferation were the Th-B peptides,
T1SP10IIIB(A) and T1-SP10MN(A), (Table 8), and

5   Candida albicans antigen (Greer Laboratories, Inc.
Lenoir, NC). PHA (Burroughs Wellcome, Research
Triangle Park, NC) was used in a wide dose range
as a mitogen in 3 day PBMC ^{3}H-thymidine
incorporation assays (Palker e. al, J. Immunol.

10   142:3612 (1989); Hart et al, J. Immunol. 145:2697
(1990)). Δcpm = experimental cpm - control cpm.
         Immunization Schedule: Because of
previous studies demonstrating the immunogenicity
of Th-B peptides in goats and rhesus monkeys (Hart

15   et al, J. Immunol. 145:2697 (1990)), the initial
comparison of peptide designs when this study
began in 1989 was monthly injections of Th-B
versus F-Th-B peptides (Table 8) at a dose of
approximately 0.1 mg/kg (6 mg/animal). When

20   neither peptide design induced neutralizing
anti-HIVIIIB antibodies, the peptide doses were
increased to approximately 0.5 mg/kg (30
mg/animal) and the right-hand side neutralizing
sequence of HIVIIIB gp120 V3 loop (the (A) region)

25   (Hart et al, Proc. Natl. Acad. Sci. USA 88:9448;
Rusche et al Proc. Natl. Acad. Sci. USA 85:3198))
(Table 8) was added to the Th-B peptide to enhance
the ability of this peptide to induce anti-HIVIIIB
neutralizing antibodies. After 3 monthly

30   injections with either ~0.5 mg/kg (30 mg) Th-B or
F-Th-B peptide, the animals were rested for 6
months, and then reimmunized with either F-Th-B or
Th-B with sequences from HIVIIIB, or with the Th-B
peptide containing HIV env gp120 V3 sequences from

35   the HIVMN isolate.
         Flow Cytometry: Chimpanzee PB
mononuclear cells were studied by standard flow

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cytometry methods using a flow cytometer (751;
Coulter Electronics, Inc., Hialeah, FL). PB
lymphocytes were identified by the following
markers; total T cells, CD3; T cell subunits, CD4

5   and CD8; B cells, CD19; and NK cells, CD56 and
CD16.

Example 2

Immunogenicity of Th B and F Th B Peptides
in Chimpanzees and Goats for Anti-Peptide and
10   Anti-HIV gp 120 Antibody Responses

For chimpanzees immunized with HIVIIIB
Th-B peptides (chimpanzee nos. 884 and 1028),
antibody to immunizing peptide rose during the
initial immunization period (Table 9). Chimpanzee
15   no. 1028 developed an abscess at the immunization
site, did not receive the month 5 immunization,
and all subsequent immunizations after month 5 in
animal 1028 were in PBS alone. Whereas peak end-
point ELISA anti-peptide antibody titer at month 4
20   in animal 1028 was 1:819,200, antibody titers fell
in animal 1028 after IFA was deleted from the
immunogen, and remained low throughout the
remainder of the immunization period (Table 9).
In chimpanzee no. 884, antibody titers rose at
25   month 7 to 1:204,800 after 5 immunizations with
Th-B peptides. Continued immunization of animal
884 with high doses of Th-B peptide (30 mg/dose)
resulted in no further increases in antibody titer
(Table 9).
30   In contrast, anti-peptide antibody
levels were much lower during months 1-10 of
immunization of animals 1045 and 1070 with HIVIIIB
F-Th-B peptide, with peak antibody levels against
immunizing peptide of 1:25,600 and 1:12,800 at

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month 7 for animals 1028 and 1070, respectively
(Table 9). After a 6 month rest for all four
animals, animals 884 and 1028 were immunized at
month 14 with 6 mg of Th-B peptide. In chimpanzee

5   no. 884, boosting with Th-B peptide in IFA at
month 14 resulted in rise in titer of anti-peptide
antibody to 1:102,400, while boosting of animal
1028 with peptide in PBS alone led to no antibody
rise (Table 9).

10   In contrast, animals 1045 and 1070 were
immunized at month 14 with 1 mg (~0.016 mg/kg) of
F-Th-B to determine if the prior doses of F-Th-B
peptide were excessive and induced high zone
tolerance, and if smaller amounts of F-derivatized

15   peptide would be more immunogenic. Immunization
of both chimpanzee nos. 1045 and 1070 with 1 mg of
F-Th-B peptide after a 6 month rest resulted in
only minimal rises in serum titers of anti-peptide
antibody to 1:800 (Table 9).

20   To determine if chimpanzees 1045 and
1070 were tolerant to Th-B peptides, both animals
were immunized on month 16 with HIVIIIB Th-B
peptide, T1-SP10IIIB(A). Both animals 1045 and
1070 responded minimally to boosting with Th-B

25   peptide with an antipeptide antibody responses to
1:1600 and 1:3200, respectively, demonstrating
that animals 1045 and 1070 were hyporesponsive at
month 16 to Th-B HIV env epitopes (Table 9).

                            Example 3

30   Immunization of Animals 1045 and 1070 with
            HIVMN Th-B Peptide Induced High Levels of
                      Antipeptide Antibodies

                Using a previously described strategy of
        breaking B cell tolerance by immunization with an

immunogen that is different from, but structurally
r lated to, the tolerogen (Weigle, Natural and
Acquired Immunologic Unresponsiveness (1967)
Chapter 4, pp. 57-151), animals 1045 and 1070
5   were next immunized with the HIVMN Th-B peptide.
The TH-B peptide from HIVMN contained the same Th
(T1) gp 120 sequence as the HIVIIIB Th-B peptide,
but contained different B cell gp 120 V3 B cell
epitope sequences than those in the HIVIIIB Th-B
10   peptide (Table 8). After 2 immunizations with
Th-B of HIVMN, beginning at month 17, both
chimpanzee nos. 1045 and 1070 had prompt rises in
titer of antibodies to HIVIIIB (Table 9) and to
HIVMN Th-B peptide (not shown) to antibody levels
15   that were higher than had previously been obtained
during the prior 18 months of study. At month 20,
endpoint ELISA titers to the HIVMN Th-B peptide
were 1:102,400 for animal 1045 and 1:204,800 for
animal 1070.

20   Example 4

              Chimpanzee B Cell Antibody Responses
              to Recombinant HIVIIIB gp120 During the
                  20-Month Immunization Course

              Endpoint ELISA antibody titers against
25   recombinant HIVIIIB gp 120 were determined for
sera from months 4-7 and 16-20 to correlate peak
anti-peptide antibody levels with anti-gp120 HIV
envelope antibody levels. It was found that peak
anti-gp120 antibody levels in chimpanzee nos. 884
30   and 1028 during months 4-7 were both 1:25,600,
whereas peak titers to gp120 in animals 1045 and
1070 during the same period were 1:6,400 and 0,
respectively. As with anti-peptide antibody
levels, boosting after a 6 month rest with peptide

in PBS in chimpanzee 1028 did not boost anti-gp120
antibodies.

        Boosting with F-Th-B peptide at month 14
and with HIVIII TH-B at month 16 in animals 1070
5   and 1045 resulted in minimal rises in anti-gp120
antibody titers by month 17 (to 1:12,800). In
contrast, boosting chimpanzees 1045 and 1070 with
HIVMN Th-B peptide at month 17 induced high levels
of anti-gp120IIIB antibody in both animals
10 (1:102,400 and 1:51,200, respectively) by month 20
that rose coincident with rises in levels of anti-
peptide antibody.

                        Example 5

                Induction of Anti-Peptide and
15   Anti-gp120 PBMC Proliferative Responses
                    by HIV Env Peptides

        Whereas HIVIIIB Th-B peptides induced
high levels (>100,000 Δcpm/10^6 cells) of PBMC
^{3}H-thymidine incorporation (animals 884 and 1028)
20 (Figures 11A and 11B) during months 1-8, F-Th-B
peptide did not induce levels of ^{3}H-thymidine
incorporation above 100,000 Δcpm/10^6 cells during
the same period (Figures 11C and 11D).
Immunization of animals 1045 and 1070 with Th-B
25 peptide at month 16 did not induce the presence of
circulating PBMC capable of proliferating to Th-B
peptide in vitro (Figures 11C and 11D).
        Interestingly, Th-B peptides at month
14-18 boosted PBMC proliferative responses in
30 animal 1028, while anti-peptide antibody responses
in animal 1028 during this time were not boosted
(Figure 11B and Table 8).
        Next, ^{3}H-thymidine incorporation of
chimpanzee PBMC to either recombinant gp120IIIB or

to native gp120IIIB was tested. Table 4 shows the
peak ^{3}H-thymidine incorporation of chimpanzee PBMC
to HIVIIIB gp120 for each animal during months
1-13, and demonstrates that neither chimpanzee no.
5   1070 nor 1045 (receiving F-Th-B peptide) had PBMC-
proliferative responses to gp120 of greater than
E/C>2 throughout the first 13 months of study. In
contrast, animals 884 and 1028 (receiving Th-B
peptides) did have anti-gp120 proliferative
10   responses during the same period (Table 4).
            To determine if PBMC proliferative
responses to mitogenic or antigenic stimuli other
than HIV immunogens were normal in the F-Th-B-
immunized chimpanzees over the 20 months of study,
15   we also measured PBMC proliferative responses to
PHA were also measured (Figure 12) and to Candida
(Figure 13). While peak PHA PBMC proliferative
responses were nearly identical in the four
chimpanzees, Candida PBMC-proliferative responses
20   varied from animal to animal and from month to
month. However, in animals 1045 and 1070, it was
found that Candida responses were intermittently
present during the time of immunization with
F-Th-B peptide at levels that were similar to
25   levels present before the immunizations were begun
(Figures 13C and 13D).

                      Example 6

              Characterization of PB Lymphocyte
           Subsets During Immunization of Chimpanzees
30   With HIV Env Peptides

            To determine if immunization with either
HIV env peptide type had effects on the number of
circulating chimpanzee T, B or NK cell
populations, the absolute numbers of these cell

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types were determined throughout the immunization
period (Figure 14, Table 10). Whereas
preimmunization (before) and postimmunization
(during) lymphocyte levels in animals 884 and 1028

5   were not significantly different (Table 10),
animal 1045 became relatively lymphogenic
(p<0.001) during the course of immunization with
F-Th-B peptide with the lymphocyte count $650/mm^3$
at week 12, compared to preimmunization levels of

10 2815 and 2597 lymphocytes/mm^3 in months 1 and 2,
respectively (Figure 14C). Whereas T cell levels
significantly dropped an average of 59% and 44% in
chimpanzee nos. 1045 (p>0.001) and 1070 (p>0.02),
respectively, during the immunization period, T

15 cell levels did not significantly change in
animals 884 and 1028 during the same time (p>0.1)
(Table 10). B and NK cell levels dropped
significantly in animal 1045, but did not change
in animals 1070, 884 and 1028 (Table 10). Taken

20 together, these data demonstrated that
immunization with the F-derivatized HIV env
peptide induced decreases in absolute levels of
circulating T cells in both animals 1045 and 1070,
and in B and NK cell levels in animal 1045,

25 whereas immunization of chimpanzee nos. 884 and
1028 with HIV Th-B env peptides lacking the F
domain did not significantly affect circulating
lymphocyte levels.

                        Example 7

30   Ability of HIVIIIB F-Th-B and Th-B
                  Peptides to Induce Anti-HIVIIIB
                  Neutralizing Antibodies in Goats

             To determine if the F-Th-B peptide used
        in the initial phase of the chimpanzee

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immunization protocol was immunogenic in another
species, 3 mg of either F-Th-B or Th-B peptide
w re used to immunize goats three times over 2
months and then used to boost goats after an 8
5   month rest (Figure 15). It was found that after
the fourth immunization, both peptides were
capable of inducing serum anti-HIVIIIB
neutralizing antibodies (Figure 15), and capable
of inducing high levels ($\geq 500,000$ Δcpm/120^6 cells)
10   of PBMC ^{3}H-thymidine incorporation in vitro to
Th-B or F-Th-B peptides. In addition, serum
endpoint ELISA titers of antibodies to immunizing
peptide were the same in Th-B and F-Th-B-immunized
goats. Thus, failure of the F-Th-B peptide to
15   induce high levels of anti-peptide antibodies and
PBMC-proliferative responses in chimpanzees was
not due to lack of an inherent immunogenicity of
the HIVIIIB F-Th-B peptide, but rather was due to
a specific effect of the F-derivatized peptide in
20   chimpanzees.

Example 8

HIVMN Th-B Env Peptide Induced
Anti-HIV Neutralizing Antibody
in Chimpanzees

25   During the first 17 months of the
immunization trial, serum-neutralizing antibodies
against HIVIIIB were always undetectable in
syncytium inhibition assay and were $\leq$ 1:45 in
reverse transcriptase inhibition assay. However,
30   following immunization of animals 1045 and 1070 at
month 17 with HIVMN Th-B peptide, anti-HIV
neutralizing antibodies were seen in syncytium
inhibition assay (Table 11).

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To determine why antibodies against
HIVIIIB Th-B peptides did not neutralize HIVIIIB
in vitro during the first 17 months of
immunization, sera from the early peak anti-
5   HIVIIIB peptide antibody responses (month 6) were
assayed for reactivity to the individual epitopes
of the Th-B peptides. It was found that at the
time of initial titers of anti-Th-B peptide
responses, most of the antibody reactivity in sera
10   from animals 884 and 1028 was indeed directed to
the primary amino acid sequence of the
neutralizing V3 loop region defined by the peptide
(TRKSIRIQRGPGR) (Table 12,). These data indicate
that antibodies made by chimpanzee nos. 884 and
15   1028 at 7 months after immunization with the
HIVIIIB Th-B HIV env peptides did not recognize
the appropriate secondary V3 loop structure(s)
necessary for neutralizing HIVIIIB, although the
animals did make antibody responses to the correct
20   primary amino acid sequences of the neutralizing
V3 B cell determinant of HIVIIIB gp120.

Example 9

Regarding the induction of tolerance, additional
clinical syndromes that might be treated using
25   Fusion domain or Fusion domain-like peptides
synthesized N- or C-terminal to an otherwise
immunogenic antigen is in hypersensitivity to bee
or wasp venom antigens and hypersensitivity to
plant or animal allergens. The nucleotide and
30   amino acid sequences of a number of allergens have
now been synthesized, and those regions of the
allergen proteins that induce IgE antibodies or T

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helper cell responses that help to induce IgE

responses are being mapped. Thus the primary

structur of grass pollen (Silvanovich et al J.

Biol. Chem. 266:1204-1220, 1991; Griffith et al

5   FEBS Letters 279:210-215, 1991; Perez et al J.

Biol. Chem. 265:16210-16215, 1990; Singh et al

Proc. Natl. Acad. Sci. USA 88:1384-1388, 1991),

mite allergens (Tovey et al J. Exp. Med. 170:1457-

1462, 1989; Yasel et al J. Immunol. 148:738-745

10   1992; Chua et al J. Exp. Med. 167:175-182, 1988;

Chua et al Int. Arch. Allergy Appl. Immunol.

91:118-123, 1990), hornet venom (Fang, et al Proc.

Natl Acad. Sci., USA 85:895-899, 1988), and tree

pollen (Ebner et al J. Immunology 150:1047-1054,

15   1993; Jarolim et al Int. Arch. Allergy Appl.

Immunol. 90:54-60, 1989; Valenta et al Science

253:557-560, 1991). For some of these allergen

proteins, T cell epitopes have been mapped (Ebner

et al J. Immunology 150:1047-1045, 1993) while for

20   others, likely T cell sites and hydrophilic B cell

determinants can be predicted using computer

algorithms (Kyte and Doolittle J. Mol. Biol.

157:105-132, 1982: Rothbard and Taylor EMBO J.

7:93, 1988; Margalit et al J. Immunol. 138:2213,

25   1987) and tested by synthesizing peptides and

injecting animals, or by reacting patient serum

antibodies or peripheral blood T cells with

synthesized peptide in in vivo assays. Once

indentified, T and B cell epitopes of bee, wasp or

other allerg ns can be synthesized with a F domain

or F-domain-lik peptide N- or C-terminal to th

allergenic T or B cell peptide, and the F-Allergen

5   epitope hybrid peptide used to inject into

patients that are sensitive to the allergen

epitope. By this method, a patient can be made

tolerant to the allergen epitope in the same

manner as chimpanzees were made tolerant to T1-

10   SP10 HIV env peptides by immunizing them with F-

T1-SP10(A) peptide (Haynes et al J. Exp. Med. in

press, 1993). Thus, in addition to treating

autoimmune disease, F-derivatizing allergen T or B

cell immunogenic peptides could product

15   tolerogenic peptides for the treatment of allergic

diseases.

A new technology has been developed whereby

injection in vivo of cDNAs with a powerful

promoter and encoding immunogenic peptides or

20   proteins has been found to promte internalization

and expression of cDNAs in host cells (Wolff et al

Science 247:1465, 1990). Thus, the above strategy

could be performed whereby cDNAs encoding F-

derivatized peptides of autoantigens and/or

25   allergens are injected instead of the peptides

themselves, thus having the same effect as

immunizing with peptides themselves. Moreover,

the F-derivatized peptides and proteins could be

produced by recombinant DNA techniques instead of

peptide synthesis of peptide synthesis and the

same type of tolerizing immunogen obtained.

     Another use of F domain- or F-like domain

5   derivatization of peptides and proteins is to

confer upon the derivatized peptide or protein the

ability to bind to the cell membrane and enter the

cell. The fusion domain or a fusion-like domain

could be conjugated to an RNA or DNA molecule as

10   well as a protein to promote entry into cells.

The ability of a molecule to enter the cells is

important for many molecules to act

therapeutically, and can be overcome by addition

of the F domain or an F-like domain to the

15   molecule that one wanted to get inside or cells.

For example, a powerful inhibitor of cell

activation would be a peptide, RNA or DNA species

of molecule that competetively bound to an

intracellular molecule ncessary for cell

20   activation, but the peptide, RNA or DNA molecule

itself did not activate or serve the normal

function of the physiologic ligand that it was

designed to mimic. Examples of peptide, RNA or

DNA molecules that might inhibit cell activation

25   would be molecules that bound to intracellular

tyrosine kinases, tyrosine phosphatases, protein

Kinase C enzymes or G proteins, just to name a few

examples. However, for peptide, RNA or DNA

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inhibitory ligands to function as cell regulatory

agents when administered as therapeutic agents,

they must readily bind the cell without killing

the cell, and be able to enter the cell and

5   function intracellularly. It has been shown that

F-derivatization of the T1-SP10IIIB(A) peptide

with the HIV gp41 F domain promotes enhanced

binding (Table 13) and entry (Table 14) of the

derivatzied T12-SP10IIIB(A) peptide into human B

10   cells. This ability to promote entry of

derivatized molecules into the inside of cells

represents a novel drug delivery system with

potential uses for delivering virtually any type

of molecule (RNA, DNA, protein) inside cells for

15   the desired therapeutic effect. For example, F-

derivatized proteins of HIV regulatory proteins

that might bind to viral RNA but not promote

transcription of RNA thus preventing normal

binding of HIV transcription factors might be used

20   to treat HIV infections in vivo.

                        *   *   *   *   *

              All publications mentioned hereinabove

are hereby incorporated in their entirety by

reference.

25   While the foregoing invention has been

described in some detail for purposes of clarity

and understanding, it will be appreciated by one

skilled in the art from a reading of this

disclosure that various changes in form and detail

can be made without departing from the true scope

5   of the invention and appended claims.

Table 1

Examples of Autoimmune Diseaes or Disease Model Caused
By Autoreactive B Cell Responses

Disease	Pathogenic Antibody Specificity
Myasthenia Gravis (MG)	Anti-acetylcholine receptor antibodies cause weakness in MG
Juvenile Onset Diabetes Mellitus (Type 1 Diabetes)	Anti-insulin antibodies and anti-islet cell antibodies mediate islet cell destruction
Graves' Disease	Anti-thyroid stimulating hormone receptor antibodies mediate the disease
Insulin Resistance in Diabetes Mellitus	Anti-insulin antibodies prevent treatment of diabetes with insulin

Table 2

Examples Autoimmuine Diseases or Disease Models Caused
by Autoreactive T Cell Responses

Experimental autoimmune uveoretinitis (EDU)	T cell responses against retinal S antigen cause eye damage
Experimental autoimmune encephalomyelitis (EAE)	T cell responses against myelin basic protein cause neuronal damage

Table 3

Peptide Sequences Used In Chimpanzee Immunizations

F-T1-SP10IIIB(A) AVGIGALFLGFLKQIINMWQEVGKAMYACTRPNNNTRKSIRIQRGPGRAFVTI

T1-SP10IIIB KQIINMWQEVGKAMYACTRPNNNTRKSIRIQRGPG

T1SP10IIIB(A) KQIINMWQEVGKAMYACTRPNNNTRKSIRIQRGPGRAFVTI

Table 4

Tritiated Thymidine Incorporation of Peripheral Blood Mononuclear
Cells Following In Vitro Stimulation With HIV Env gp120*

Chimpanzee No.	Immunogen	Pre-Immunization	Post-Immunization (Post/Pre)
		Δcpm/10^6 cells	
884	T1-SP10IIIB, then T1-SP10IIIB(A)	169	39,189 (232)
1028	T1-SP10IIIB, then T1-SP10IIIB(A)	17,955	129,121 (7)
1045	F-T1-SP10IIIB(A)	6,348	12,256 (2)
1070	F-T1-SP10IIIB(A)	11,285	22,719 (2)

*Data represent the peak gp120 responses observed during the immunization
period. Data for animals 884,1028, and 1045 represent peak responses using
from 2ug/ml to 0.5ug/ml of HIVIIIB(LAI) recombinant gp120. Data for animal
1070 represent peak responses using from 1ug/ml to 0.5ug/ml of native
HIVIIIB(LAI) gp120.

Table 5

HIV Envelope gp41 Fusion Protein (F) Sequences From Multiple

HIV Isolates

Isolate	Sequence
HIV-1	
BH10	A V G : I G A L F L G F L
MN	A A : : - - - - - - - - -
SC	- - - T - - - M - - - - -
SF2	- - - I V - - M - - - - -
CDC4	- - - M L - - M - - - - -
WMJ2	- - - T - - - M - - - - -
RF	- - - T - - - M - - - - -
ELI	- I - : L - - M - - - - -
MAL	- I - : L - - M - - - - -
Z6	- I - : L - - M - - - - -
2321	- I - M : - - P - - - - -
JY1	- I - : L - - V - - - - -
WMJ-1	- - - A - - - M - - - - -
HIV-2	
ROD	R G V F V L G F L G F L
NIHZ	- - - - - - - - - - - -

Sequences for BH10 are aa 519-530 from Ratner, L, et al.
Nature 313: 277-284, 1985. Sequences for the remainder of the
HIV-1 and HIV-2 isolates from Myers, et al. Human
Retroviruses and AIDS, 1988, Los Alamos National Laboratory,
Los Alamos, New Mexico, p. II-90. WMJ-1 sequence from ref.
18.

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Table 6

Regions of the TSH Receptor to Which Patient
Anti-TSH Receptor Autoantibodies Bind

Amino Acid No.	Sequence	Ref.
333-343	YVFFEEQEDEI	17
12-36	HQEEDFRVTCKDIQRIPSLPPSTQT	18
289-317	LRQRKSVNALNSPLHQEYEENLGDSIVGY	18
352-366	YYVFFEEQEDEIIGF	27
103-111	YKELPLLKFL	28

Amino acid numbers and sequence from the reference listed

Table 7

Examples of Hybrid Peptide Constructs That Could Be Used To
Treat Anti-HLA Immune Responses In AIDS

<u>HIV gp120 homology with DP/DQ β chain</u>
gp120 aa261-270   VVSTQLLLNG
HLA DP/DQ aa142-151   VVST*LI*NG

<u>HIV gp41 homology with HLA DR β chain</u>
gp41   aa837-844   EGTDRVI
HLA DR aa19-25   NGTERVR

<u>Hybrid Immunogens</u>:   AVGIGALFLGFLVVSTQLLLNG
                       AVGIGALFLGFLVVSTLING
                       AVGIGALFLGFLEGTDRVI
                       AVGIGALFLGFLNGTERVR

HIV gp120 and gp41 homologies with HLA Class II are from refs. 25
and 26.

TABLE 8

Sequences of Synthetic Peptide Constructs
Derived From HIV MN and HIVIIIB Env gp120*

Peptide Name	Peptide Type	Peptide Composition and Sequence (Epitope Type)			
		F	T1(Th)	SP10(B cell)	A(B cell)
F-T1-SP10IIIB(A)	F-Th-B	AVGIGALFLGFLKQIINMWQEVGKAMYACTRPNNNTRKSIRIQRGPGRAFVTI
T1-SP10IIIB(A)	Th-B	KQIINMWQEVGKAMYACTRPNNNTRKSIRIQRGPGRAFVTI
T1-SP10IIIB	Th-B	KQIINMWQEVGKAMYACTRPNNNTRKRIRIQRGPG
T1-SP10MN(A)	Th-B	KQIINMWQEVGKAMYACTRPNYNKRKRIHIGPGRAFYTTK

*Each amino acid is represented by a single-letter code that is the first letter of its name, except for arginine (R), asparagine (N), glutamine (Q), glutamic acid (E), lysine (K), phenylalanine (F), tryptophan (W), tyrosine (Y), and aspartic acid (D). F (fusogenic domain) sequence is amino acids 519-530 from HIVIIIB (27). T1 sequence is amino acids 428-443 from HIVIIIB (27). SP10MN(A) sequence is amino acids 301-319 from HIVMN (28). SP10IIIB sequence is amino acids 303-321 from HIVIIIB. (A) sequence is amino acids 320-324 from HIVMN (28) and amino acids 322-327 from HIVIIIB (27).

Th= T helper cell determinant.
B cell = B cell neutralizing antibody determinant.
A = Additional HIV gp120 V3 loop sequences added to the original synthetic peptide (SP10)
    sequence to add an additional neutralizing and CTL region to the HIV B cell determinant
    of the hybrid peptide.

27 = Ratner et al, Nature 313:277 (1985)
28 = Myers et al, Human Retroviruses and AIDS (1991), p. III 6-23

Table 19

Time Course of Anti-Peptide Antibody Responses in Chimpanzes
Immunized with HIV Envelope Synthetic Th-B or F-Th-B Peptides

Month of Study	Immunogen	Chimpanzee Number 884 Reciprocal of ELISA Titer	Chimpanzee Number 1028 Reciprocal of ELISA Titer	Immunogen	Chimpanzee Number 1045 Reciprocal of ELISA Titer	Chimpanzee Number 1070 Reciprocal of ELISA Titer
1		0	0		0	0
2	Th-B(IIIB) 6mg	0	0	F-Th-B(IIIB) 6mg	0	0
3	Th-B(IIIB) 6mg	51,200	102,400	F-Th-B(IIIB) 6mg	0	0
4	Th-B(IIIB) 6mg	25,600	819,200	F-Th-B(IIIB) 6mg	0	800
5	Th-B(IIIB) 6mg	25,600	204,800*	F-Th-B(IIIB) 6mg	1,600	200
6	Th-B(IIIB) 30mg	51,200	102,400	F-Th-B(IIIB) 30mg	25,600	12,800
7	Th-B(IIIB) 30mg	204,800	102,400	F-Th-B(IIIB) 30mg	25,600	12,800
8	Th-B(IIIB) 30mg	51,200	25,600	F-Th-B(IIIB) 30mg	6,400	12,800
9		51,200	51,200		3,200	6,400
10		12,800	25,600		800	800
11		51,200	25,600		800	1,600
12		51,200	25,600		1,600	800
13		25,600	25,600		200	200
14	Th-B(IIIB) 6mg	51,200	25,600	F-Th-B(IIIB) 1mg	200	400
15		102,400	12,800		800	800
16	Th-B(MN) 6mg	25,600	12,800	Th-B(IIIB) 6mg	100	0
17	Th-B(MN) 6mg	12,800	3,200	Th-B(MN) 6mg	1,600	3,200
18		25,600	6,400		6,400	25,600
19	Th-B(MN) 6mg	25,600	1,600	Th-B(MN) 6mg	6,400	51,200
20		51,200	6,400	Th-B(MN) 6mg	51,200	102,400#

Titers are endpoint ELISA titers (titers at which E/C were ≥ 3.0) against the Th-B peptide, T1-SP10IIIB.
* Animal 1028 did not receive the month 5 injection due to a sterile abscess at the injection site. All injections in animal 1028 after month 5 were in PBS alone.
# Animal 1070 did not receive the month 20 immunization due to the presence of high levels of anti-HIV neutralize antibodies.
For animals 884 and 1028, immunizations at months 2-5 were with T1-SP10IIIB, months 6,7,8 and 14, T1-SP10IIIB(A). For animals 1045 and 1070 immunization at month 16 was with T1-SP10IIIB(A).

Table 10.

Mean Lymphocyte and Lymphocyte Subset Levels In Chimpanzees
Before and During Immunization With HIV Envelope Synthetic Peptides*

Chimpanzee Number

Leukocyte Subset	884			1028			1045			1070		
	Before	During	% Change	Before	During	% Change	Before	During	% Change	Before	During	% Change
	Cells/mm³ ± SEM											
Total Lymphocytes	4034±452	3046±249	-26%	3164±396	3286±660	+4%	3164±397	1426±116	-55%	3943±885	2768±296	-30%
T cells	2629±384	2054±178	-24%	2565±276	2027±402	-21%	2460±253	1012±82	-59%	3337±762	1887±184	-44%
B cells	356±39	365±39	+3%	411±103	458±47	+11%	293±32	175±15	-40%	302±53	232±22	-23%
NK cells	345±82	317±43	-9%	257±25	434±128	+68%	112±27	61±7	-45%	478±148	306±44	-36%

*"Before" samples were studied over a 5 month period prior to immunization with peptides; n = 5 for lymphocytes, n = 3
for T cells, B cells and NK cells. "During" samples were taken from months 2-14 of immunization; n = 11 for
lymphocytes, T, B, and NK cells. Unless noted, P values for percent change comparing "before" values with "during"
values was not significant with p> .05 using student's t test.

* = p> .001
† = p> .02
‡ = p> .005

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Table 11

Neutralization of HIV LAI/IIIB and HIV MN in Syncytium Inhibition
Assay in Chimpanzees Immunized with T1-SP10 Peptides

	Month 18		Month 19		Month 20	
Animal No.	LAI/IIIB	MN	LAI/IIIB	MN	LAI/IIIB	MN
	Presence of Neutralization in Syncytium Inhibition Assay					
	(Reciprocal Titer in RT Inhibition Assay)					
884	-	- (20)	-	-	-	- (24)
1028	-	-	-	-	-	-
1045	-	- (23)	+/- (23)	- (23)	- (22)	- (24)
1070	+/- (92)	- (22)	+ (100)	+ (96)	+/- (86)	++ (350)

- = < 48% inhibition of syncytia.
+/- = ≈ 49% and < 80% inhibition of syncytia.
+ = ≈ 80% inhibition of syncytia, titer 1:10.
++ = ≈ 80% inhibition of syncytia, titer 1:20.

Table 12

Reactivity of Chimpanzee Serum with Truncated Forms of
the Th-B Peptide T1-SP10IIIB#

Chimpanzee No. (Bleed Date)	Peptide Used in ELISA Binding Assay				
	T1-SP10IIIB	T1-flu	SP10C	SP10D	SP10E
	Endpoint Titer (> 3.0 E/C) In ELISA Assay				
884 (Month 7)	204,800	800	> 102,400*	51,200	3,200
1028 (Month 7)	102,400	800	102,400	51,200	3,200

#Peptides used in ELISA Assay were:

T1-SP10IIIB	-	KQIINMWQEVGKAMYACTRPNNNTRKSIRIQRGPG
T1-flu	-	KQIINMWQEVGKAMYATYQRTRALVTG
SP10C	-	(C)TRKSIRIQRGPGR(Y)
SP10D	-	(C)IRIQRGPGR
SP10E	-	(C)TRPNNNTRKSIR

ELISA assay performed as described in Methods.
Flu sequence (TYQRTRALVTG) is from influenza nucleoprotein, strain A PR/8/34
from Deres et al, Nature 342:561 (1989).

*E/C at 1:102,400 = 6.0.

Table 13

Effect of Derivatizing T1-SP10IIIB(A) Peptide With
the HIV gp41 Fusogenic (F) Domain on Peptide
Ability to Bind to Human Cells

Peptide	Antibody	MFC 4 Degrees C, 1 Hr.	MFC 37 Degrees C, 21 Hr.
None	Anti-gp120	7.6	13.6
T1-SP10IIIB(A) 10ug/ml	Anti-gp120	14.7	14.0
F1-T1-SP10IIIB(A)	Anti-gp120	82.8	36.7

Anti-gp120 momoclonal antibody was 0.5beta from
the NIAID AIDS Research and Reference Reagent
Program (Matsushita et al J. Virol. 62:2107,
1988). Cells used were human JY B cells which
were incubated either for 1 hour at 4 degrees C or
for 21 hours at 37 degrees C and then reacted with
saturating amounts of the anti-gp120IIIB mab,
0.5beta followed by FITC-conjugated goat anti-
mouse Ig reagent. The amount of fluoresence was
determined on a flow cytometer and fluoresence
brightness was expressed as MFC=mean channel
fluoresence.

Table shows that conjugation of the F domain
on the T1-SP10IIIB(A) peptide confers on it the
ability to bind to JY B cells better that the T1-
SP10IIIB(A) peptide alone, and that after
incubation at 37 degrees C, the F-T1-SP10IIIB(A)
peptide is decreased on the surface of the cells.

Table 14

Reactivity of anti-gp120 Monoclonal Antibody with
Acetone-Fixed JY B Cells That Had Been Incubated
With F-T1-SP10IIIB(A) Peptide (10μg/ml) For 21
Hours at 37 Degrees C

Peptide	Antibody	% Intracytoplasmic Positive
T1-SP10IIIB(A)	Control	0
T1-SP10IIIB(A)	Anti-gp120	0
F-T1-SP10IIIB(A)	Control	0
F-T1-Sp10IIIB(A)	Anti-gp120	76 faint, 24 bright

Cells were incubated as descirbed in Table 13.
After 21 hours at 37 degrees C, cytocentrifuge
preparations of cells were prepared, acetone
fixed, and reacted either with control mab P3x63
Ag8 or with anti-gp120 mab 0.5beta. Slides were
read for either faint or bright intracytoplasmic
fluoresence on a fluoresence microscope. Data
show that after incubation of 10 ug/ml of peptide
for 21 hours at 37 degrees C, the F-T1-SP10IIIB(A)
peptide could be detected inside the JY B cells
whereas the T1-SP10MN(A) peptide could not be
detected.

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WHAT IS CLAIMED IS:

1.   A method of inducing immune
tolerance in a mammal to an immunogenic peptide or
protein comprising: administering to said mammal a
synthetic immune system toleragen comprising a 2
to 20 amino acid hydrophobic peptide linked to the
N-terminus or C-terminus of said immunogenic
peptide or protein, under conditions such that
said immune tolerance is induced.

2.   The method according to claim 1,
wherein said mammal is a primate.

3.   The method according to claim 2,
wherein said primate is a human.

4.   The method according to claim 1,
wherein the hydrophobic peptide comprises 5 to 15
amino acids.

5.   The method according to claim 2,
wherein the hydrophobic peptide comprises 7 to 13
amino acids.

6.   The method according to claim 1,
wherein the hydrophobic peptide is a segment from
a HIV or HIV-related virus protein.

7.   The method according to claim 1,
wherein the hydrophobic peptide is AVGIGALFLGFL.

8.   The method according to claim 1,
wherein the immunogenic peptide or protein is an
acetylcholine receptor protein.

9.   The method according to claim 1,
wherein the immunogenic peptide or protein is an
acetylcholine receptor protein, or fragment
thereof.

10ı   The method according to claim 1,
wherein the immunogenic peptide or protein is an
insulin protein, or fragment thereof.

11.   The method according to claim 1,
wherein the immunogenic peptide or protein is a
TSH receptor protein, or fragment thereof.

12.   The method according to claim 1,
wherein the immunogenic peptide or protein is an
autoimmune T cell antigen, or fragment thereof.

13.   The method according to claim 1,
wherein the immunogenic peptide or protein is a
retinal S protein, or fragment thereof.

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14. The method according to claim 1 wherein the immunogenic peptide or protein is a B cell determinant or a protein that induces pathogenic B cell antibody production in an autoimmune or inflammatory disease.

15. The method according to claim 1 wherein the hydrophobic part of the toleragen is any hydrophobic peptide from a transmembrane region of a transmembrane protein, or is a random mix of hydrophobic amino acids.

FIG.1A
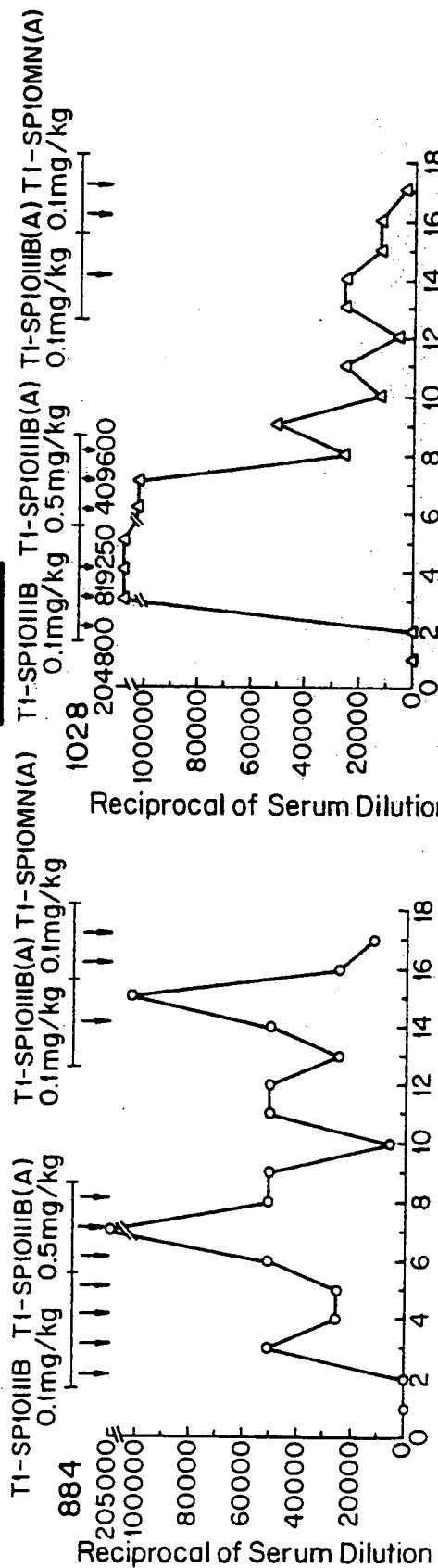
FIG.1B
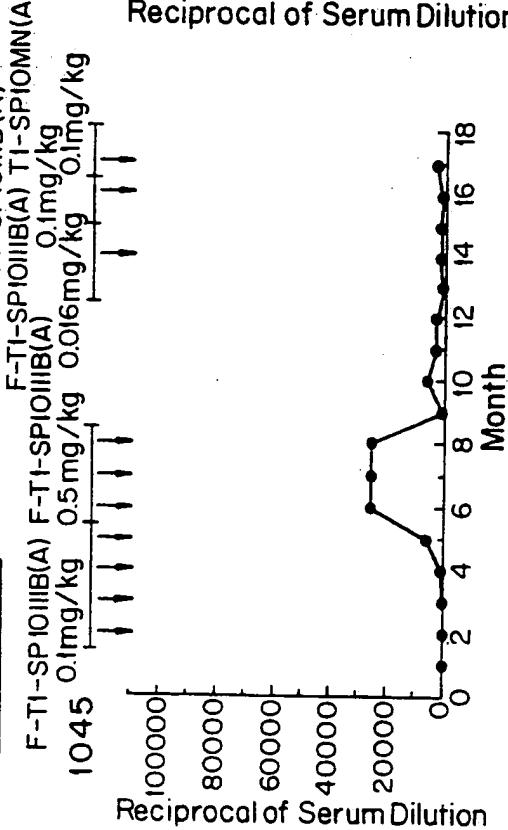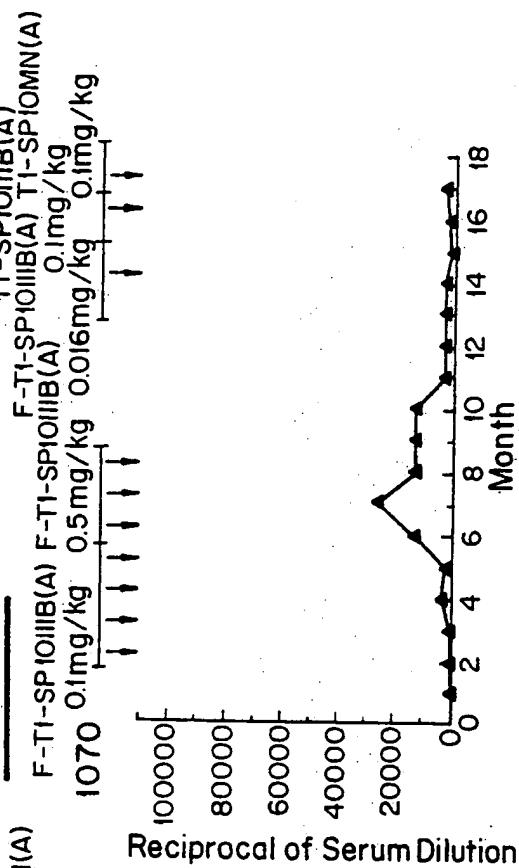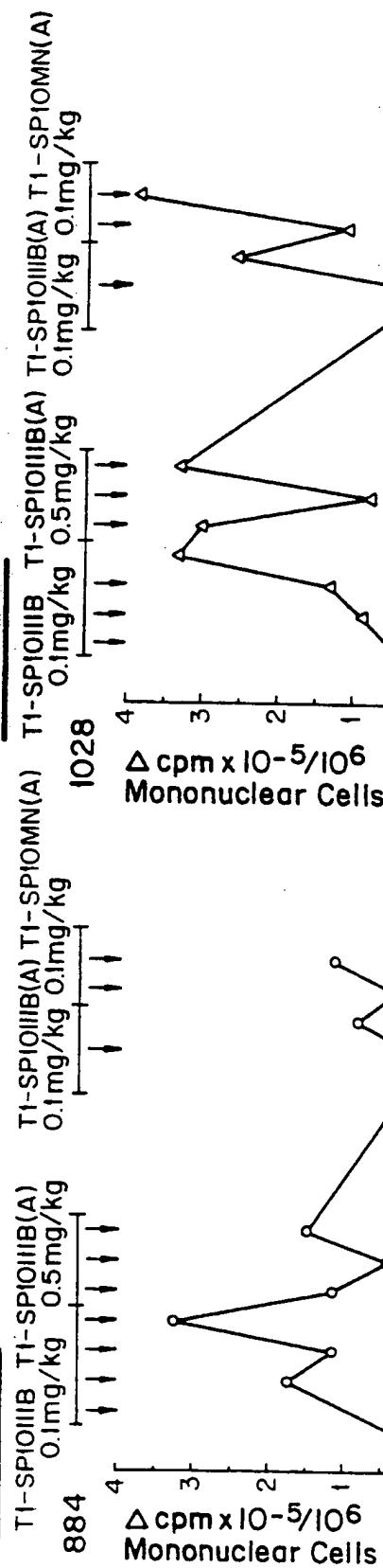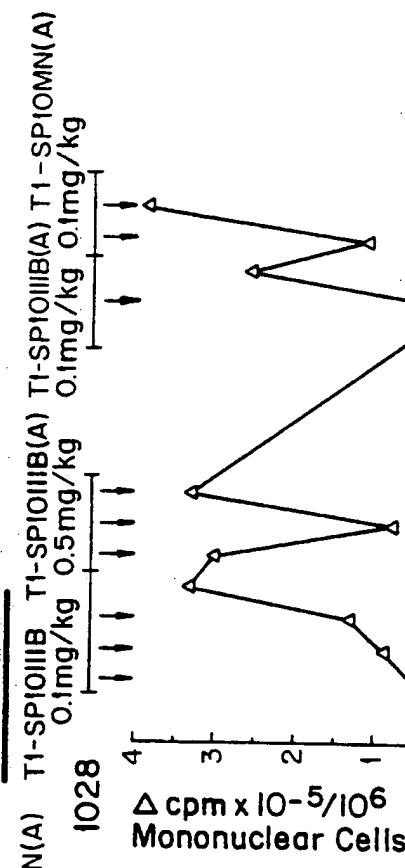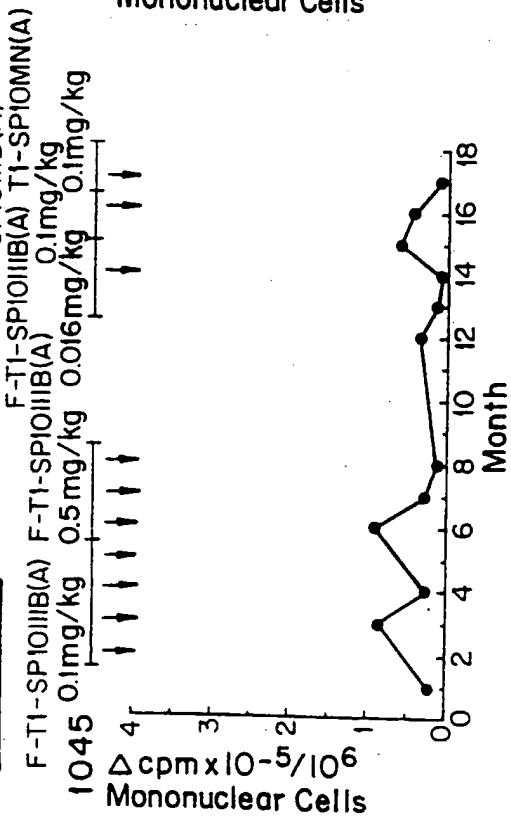
FIG.1C

FIG.1D

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FIG.2A

FIG.2B

FIG.2C

FIG.2D
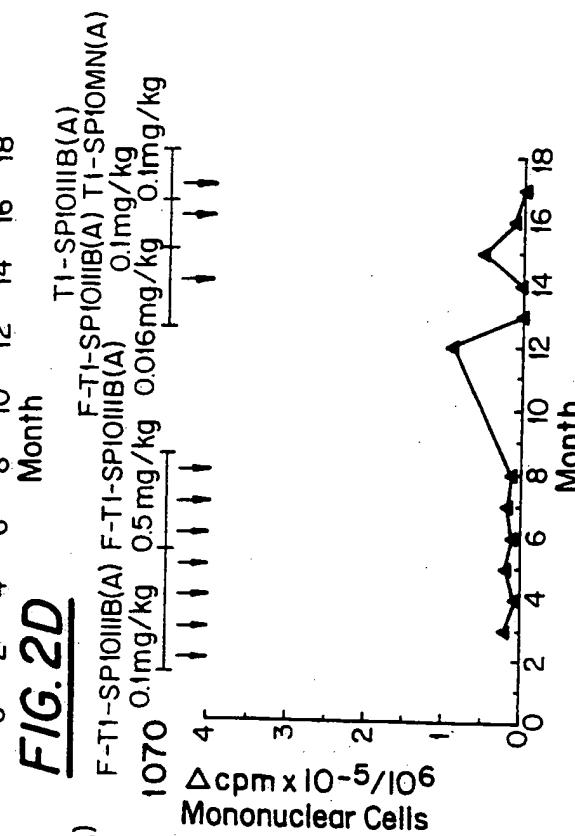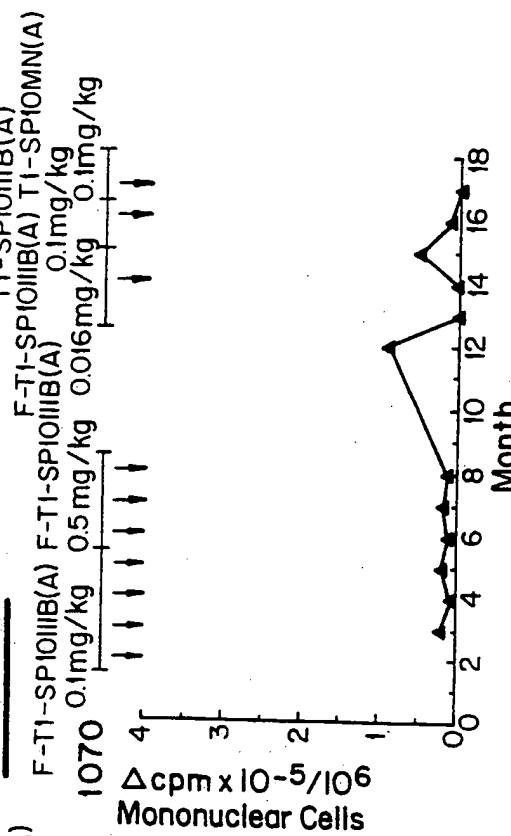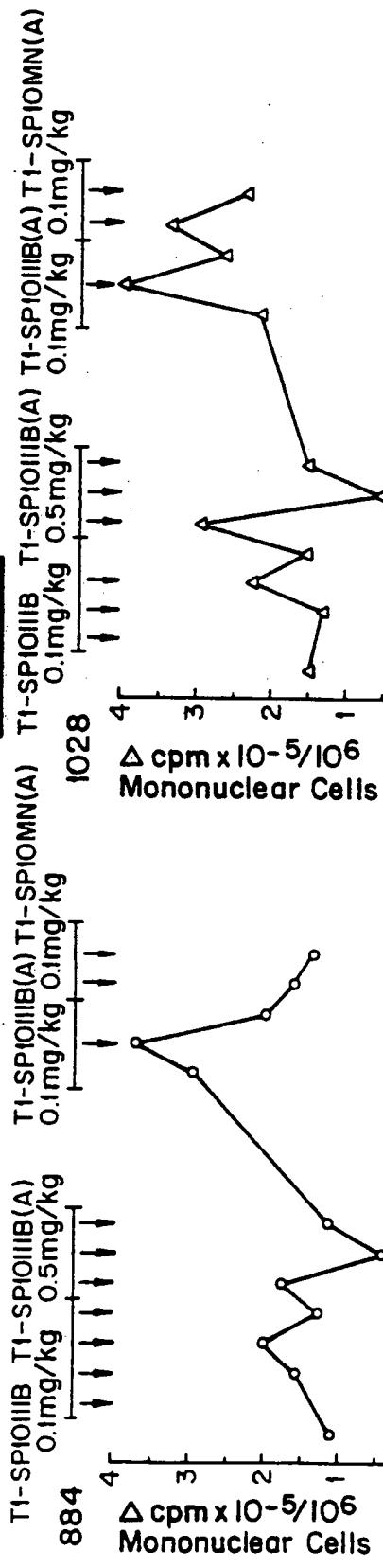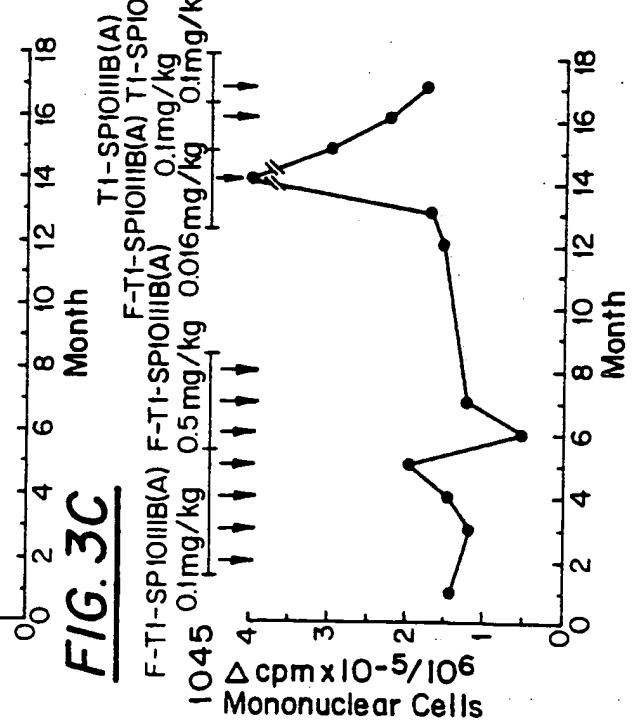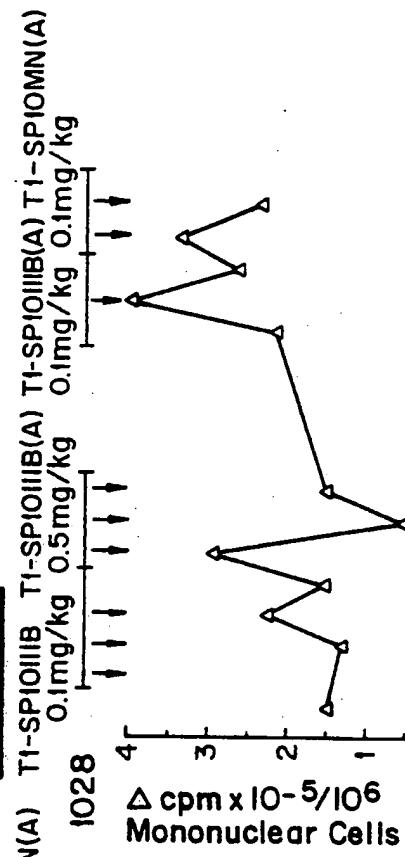
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FIG.3A

FIG.3B

FIG.3C

FIG.3D

3/14

FIG. 4

SP1OMN(A)

FIG. 5

T1-SP1OMN(A)

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FIG. 6

F-T1-SP1OMN(A)

FIG. 7

F-SP1OMN(A)
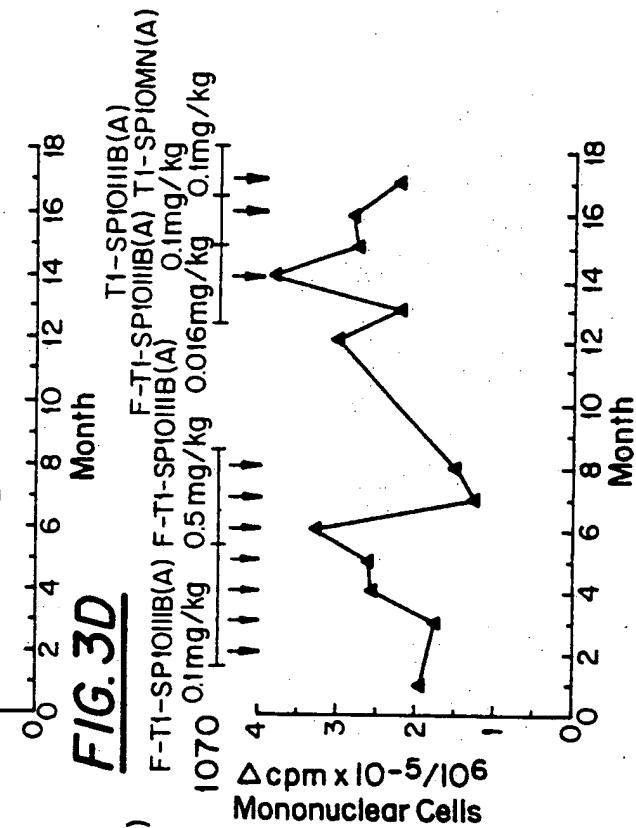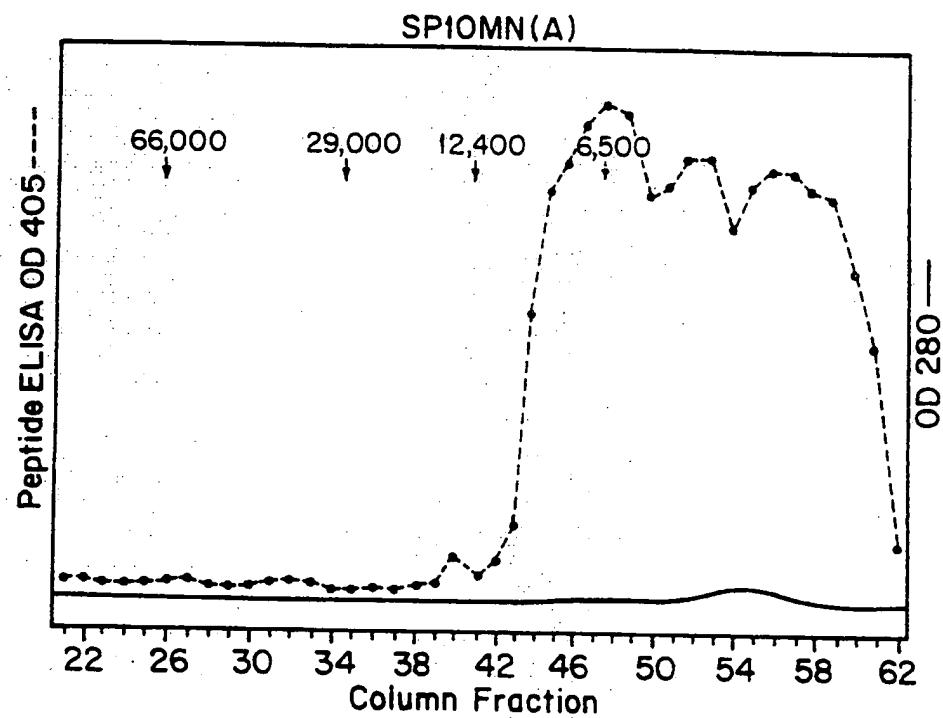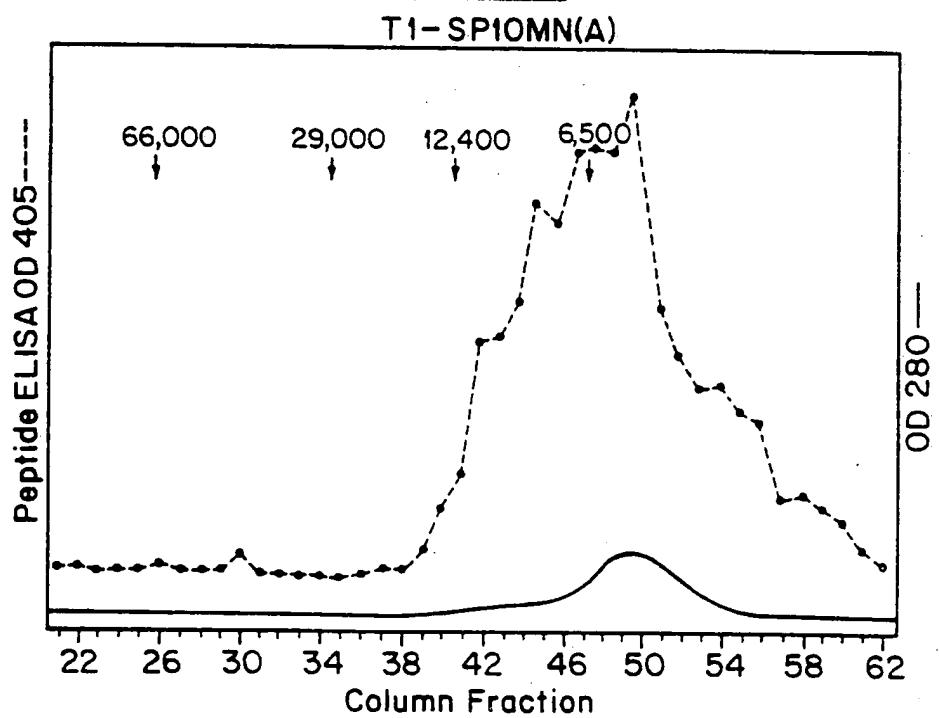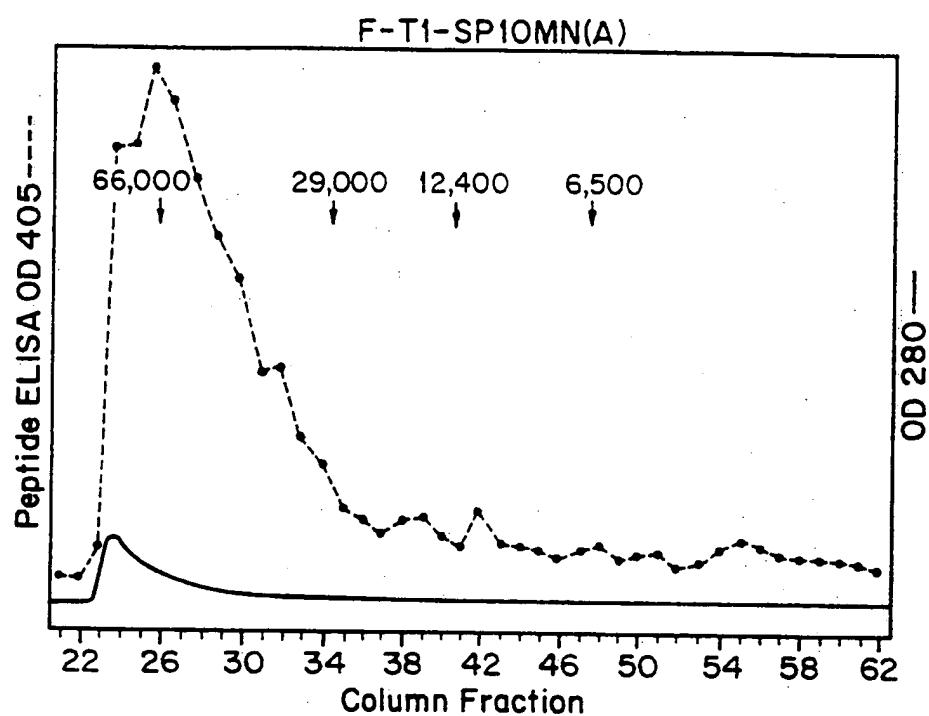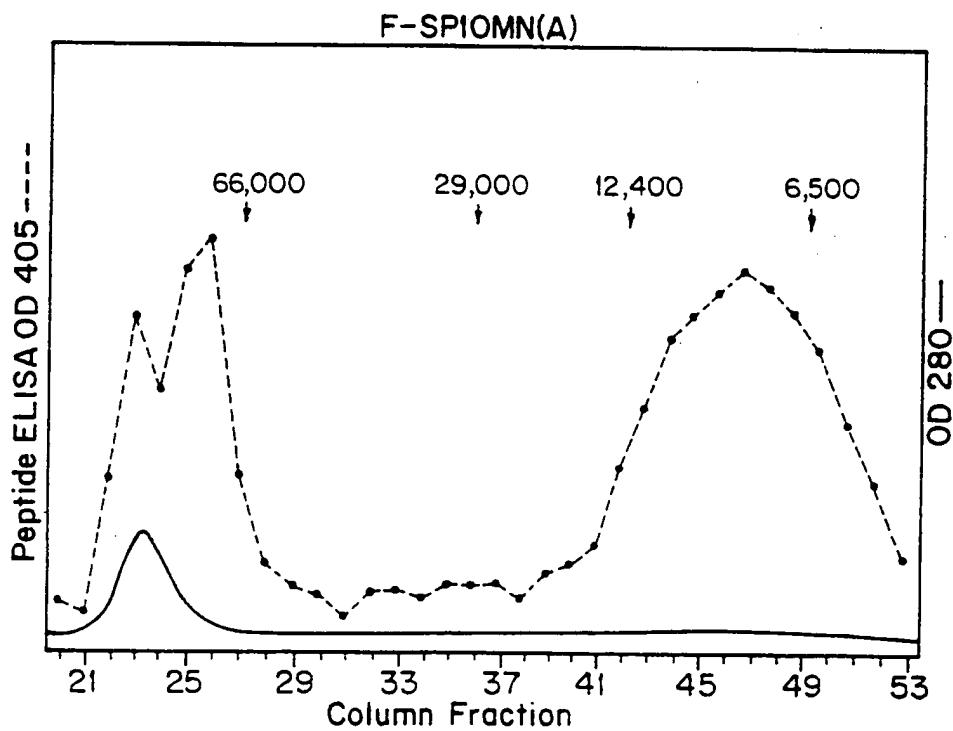
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FIG. 8
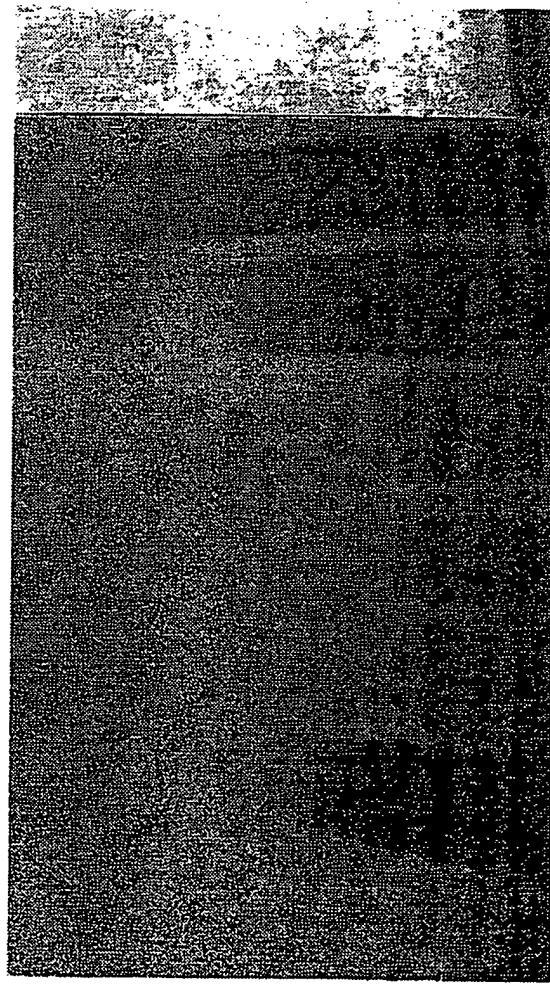
FIG. 9

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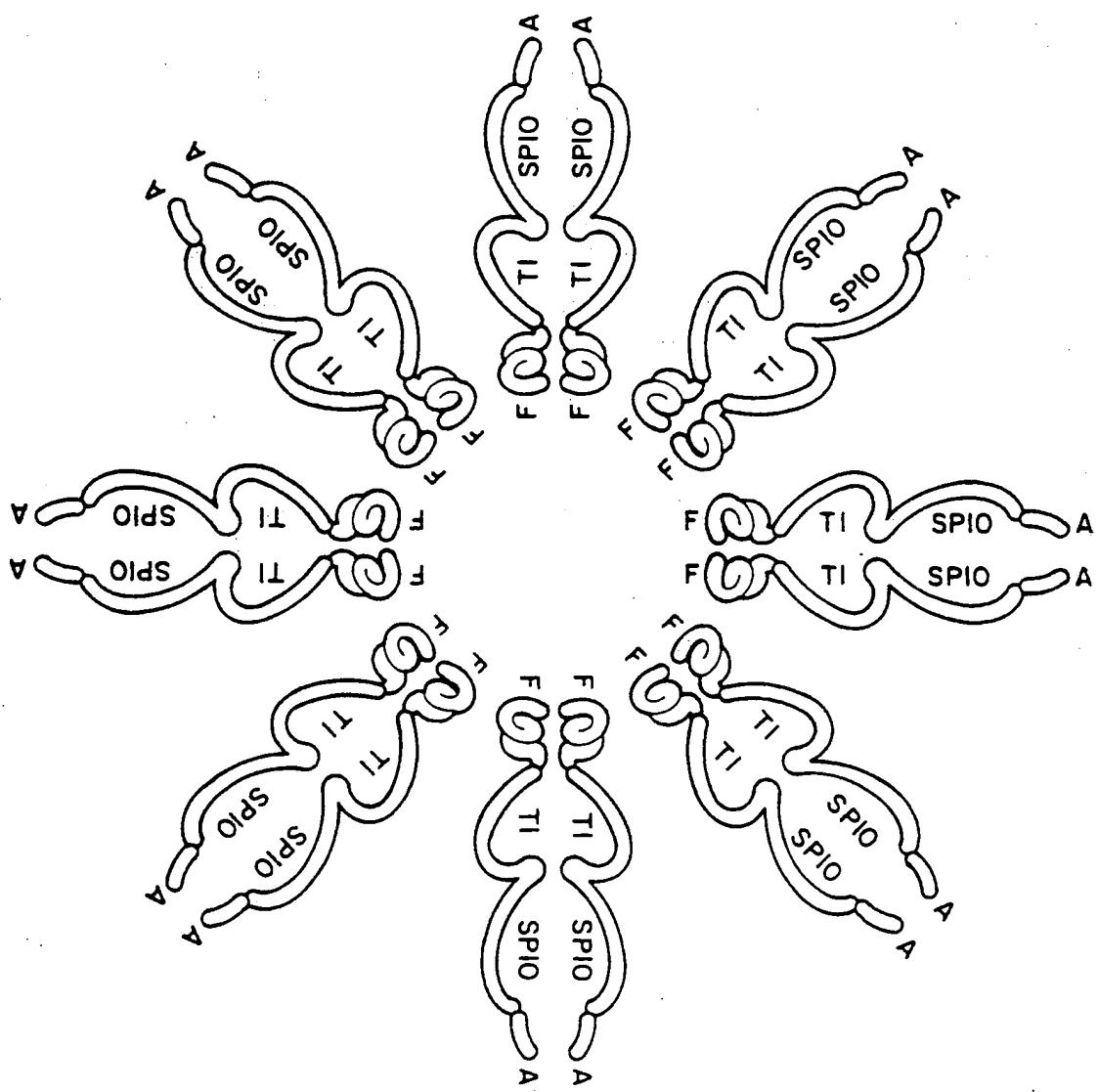
FIG. 10

Envelope Sequences   Region

Peptide Name	F	T1	SP10	A
HIV MN				
T1-SP10MN		KQIINMWQEVGKAMYACTRPNYNKRKRIHIGPGRA		
T1-SP10MN(A)		KQIINMWQEVGKAMYACTRPNYNKRKRIHIGPGRA FYTTK		
F-T1-SP10MN	AVGIGALFLGFLKQIINMWQEVGKAMYACTRPNYNKRKRIHIGPGRA			
F-T1-SP10MN(A)	AVGIGALFLGFLKQIINMWQEVGKAMYACTRPNYNKRKRIHIGPGRA FYTTK			

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FIG. 10 cont.

```
F-SP1OMN(A)        AVGIGALFLGFL..............CTRPNYNKRKRIHIGPGRA
                   FYTTK

SP1OMN(A)                                     CTRPNYNKRKRIHIGPGRA
                                              FYTTK

HIVIIIB

T1-SP10IIIB(A)                    KQIINMWQEVGKAMYACTRPNNNTRKSIRIQRGPG
                                  RAFVTI

F-T1- SP10IIIB(A)  AVGIGALFLGFLKQIINMWQEVGKAMYACTRPNNNTRKSIRIQRGPG
                   RAFVTI
```

FIG.IIA
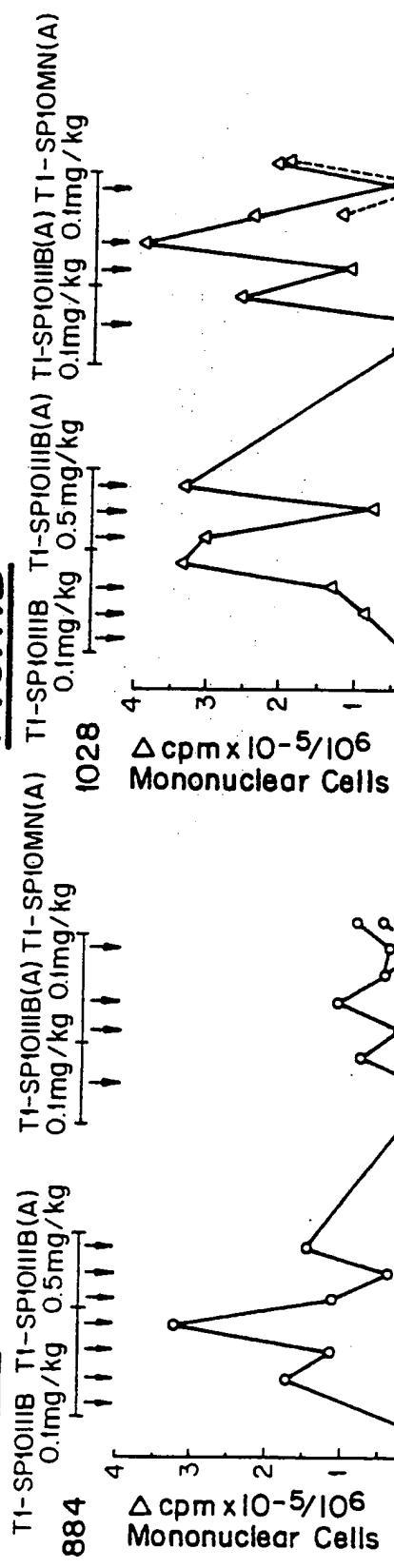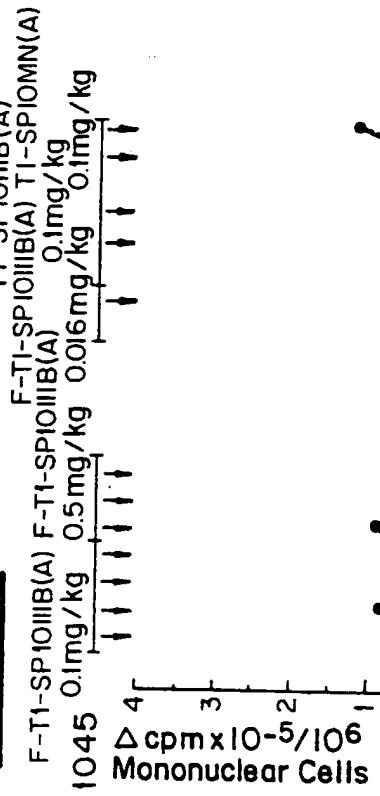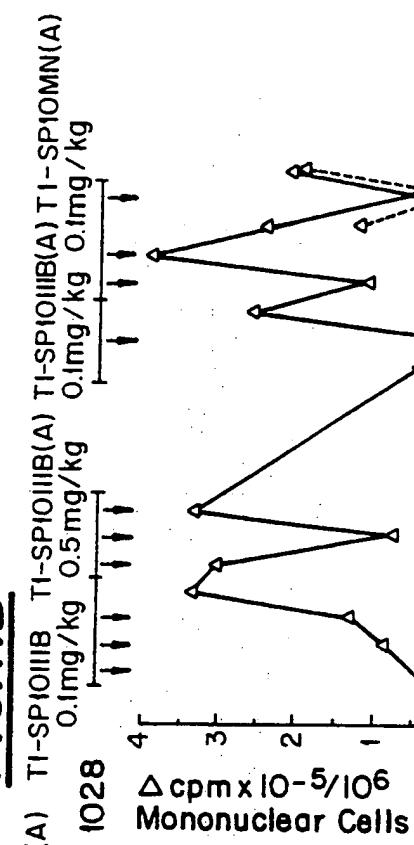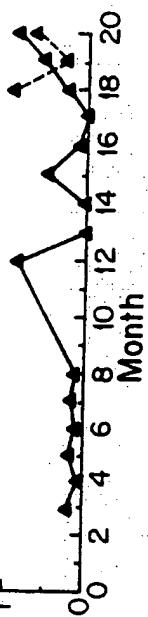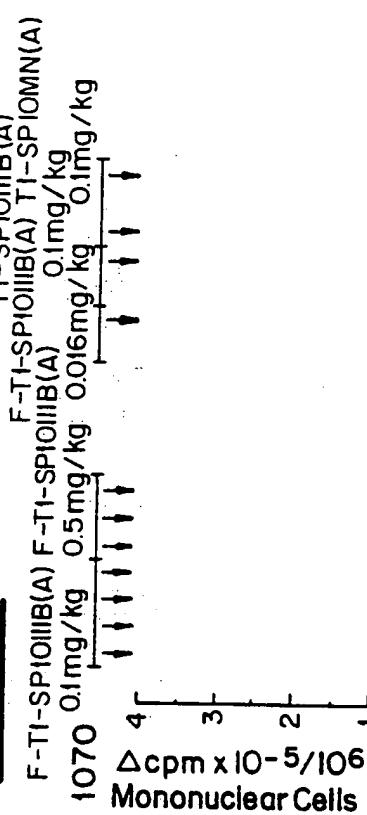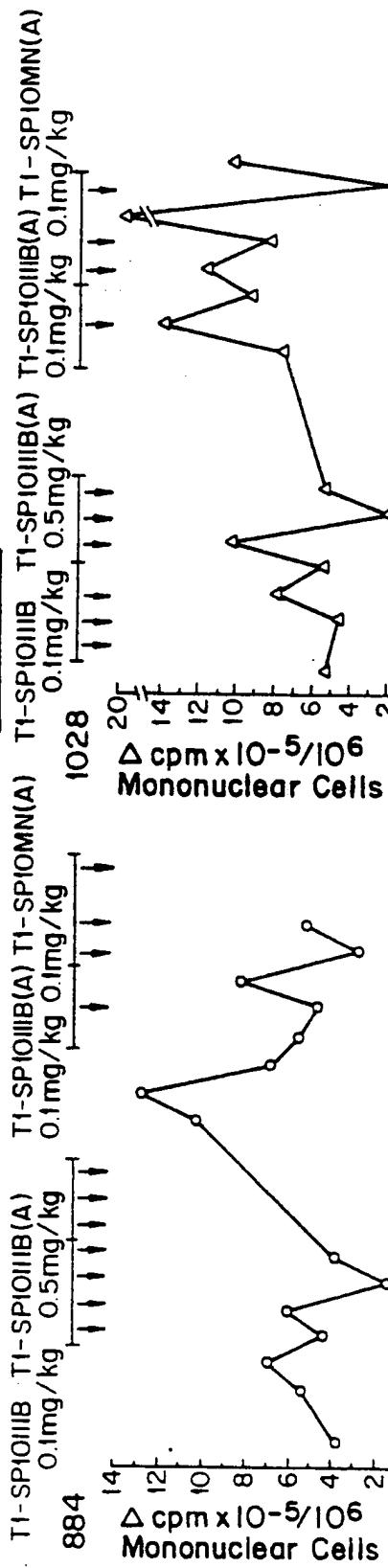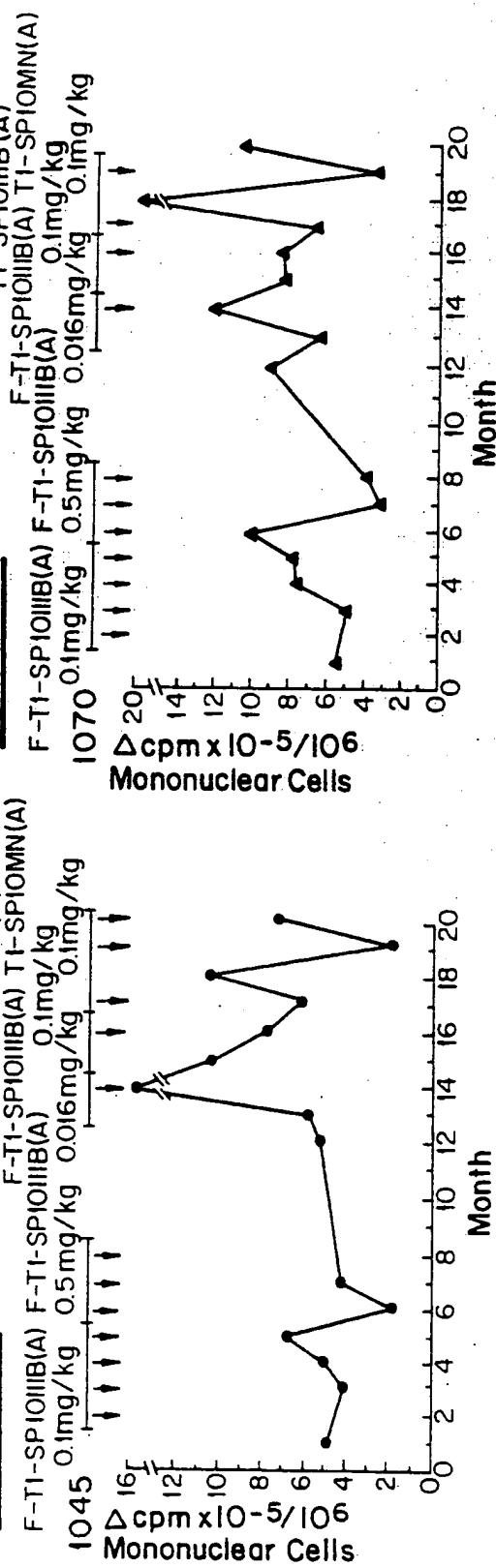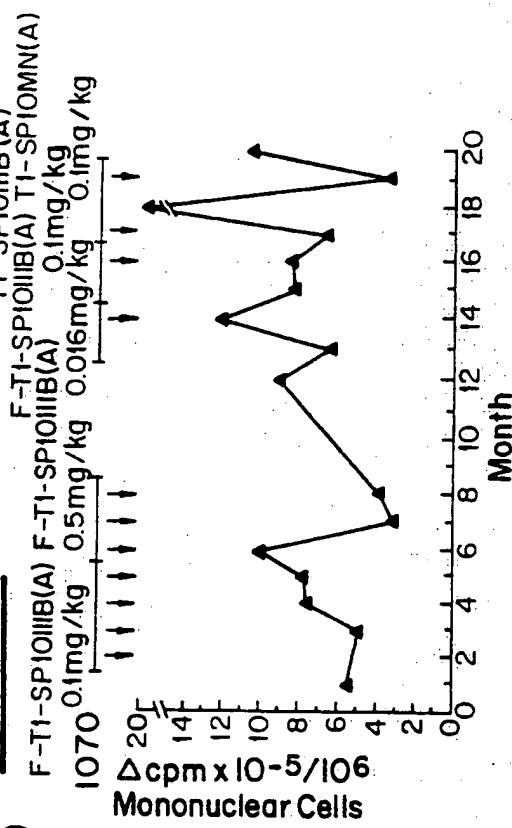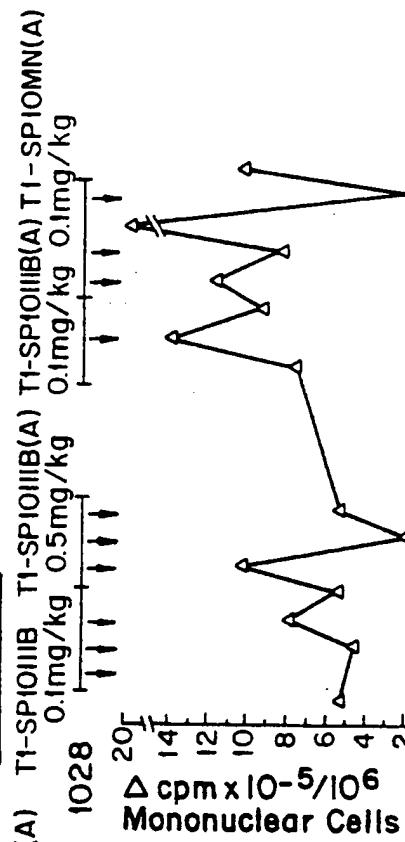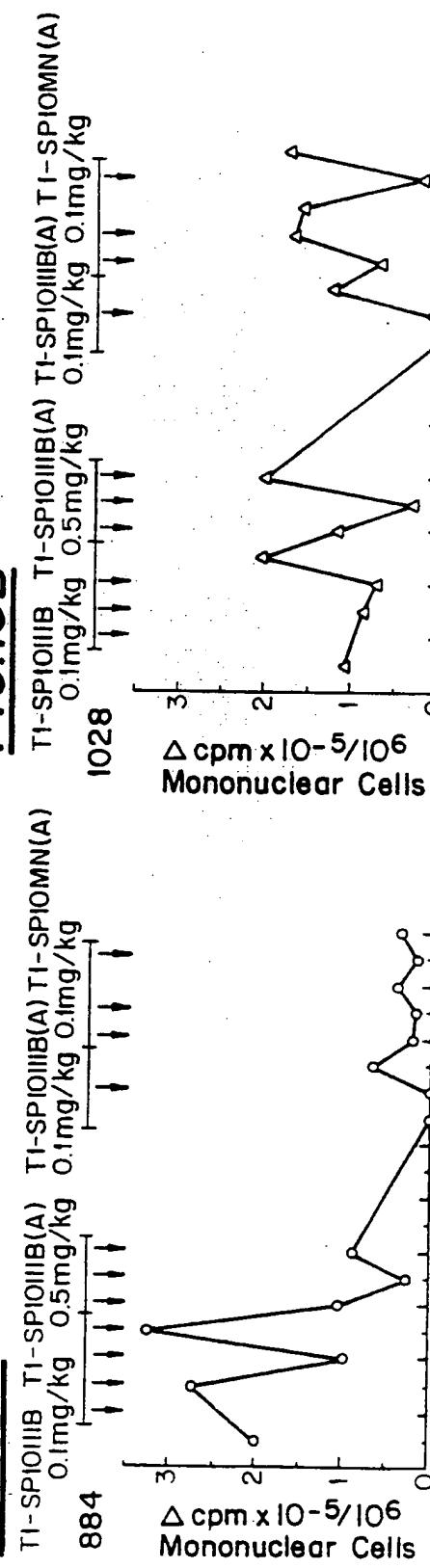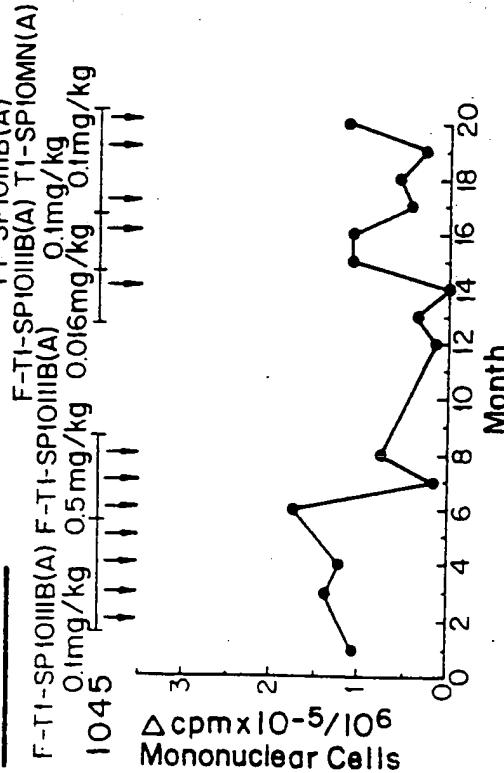
FIG.IIB

FIG.IIC

FIG.IID

FIG.12B

FIG.12A

FIG.12D

FIG.12C

FIG.13B

FIG.13A

FIG.13D

FIG.13C

FIG.14B

FIG.14A

FIG.14D

FIG.14C

FIG. 15

SUBSTITUTE SHEET
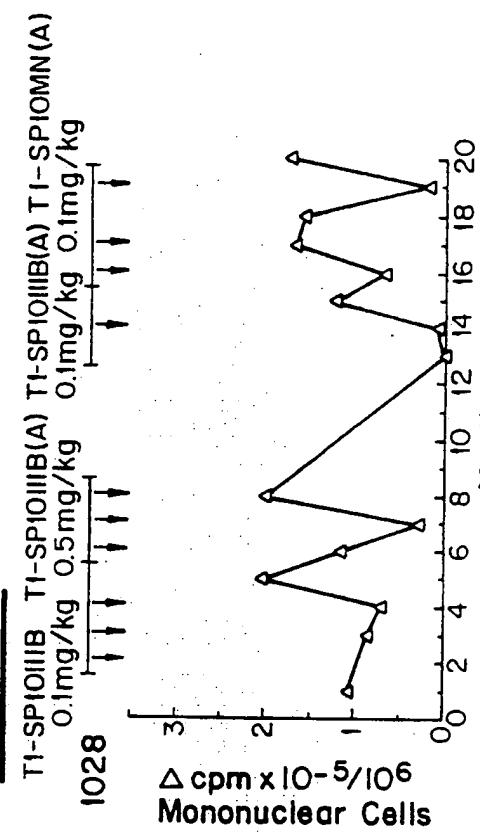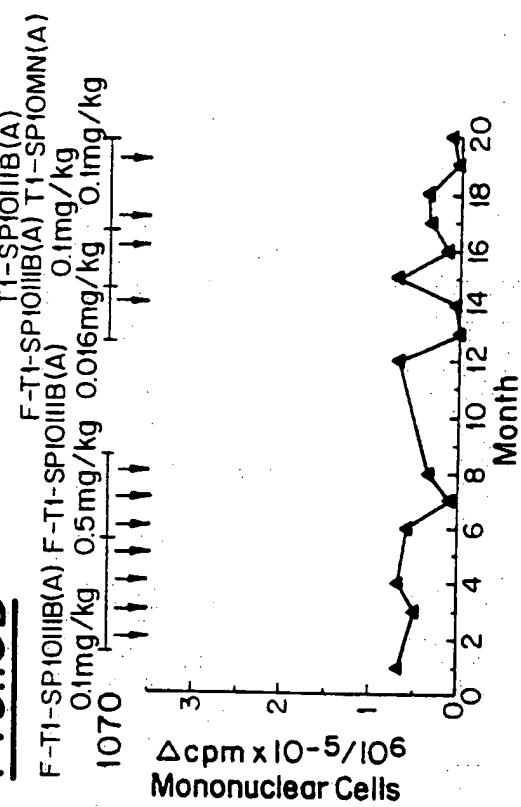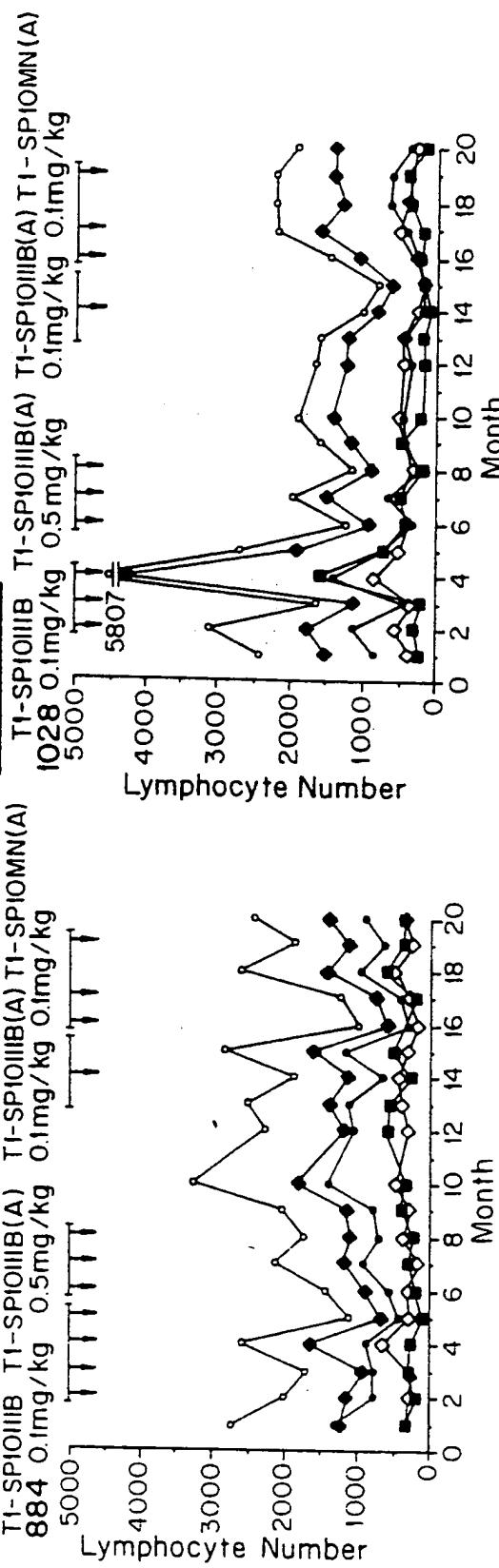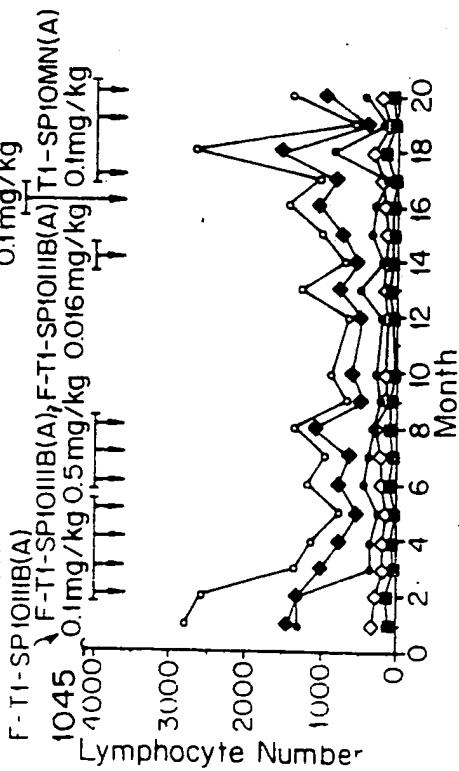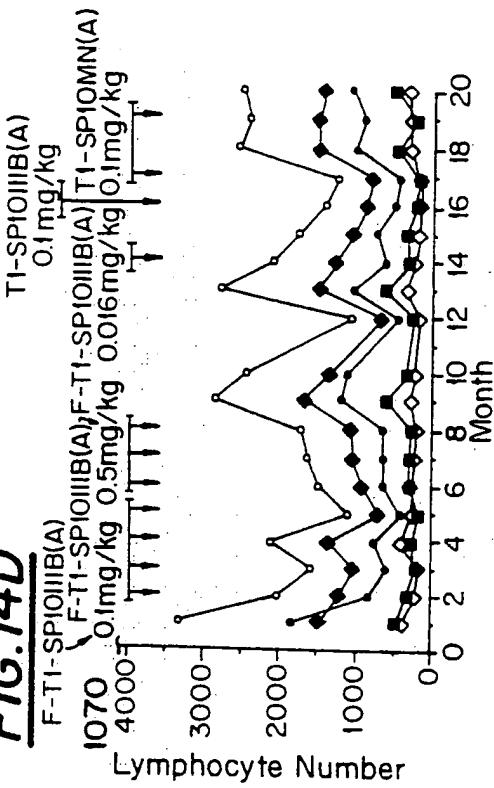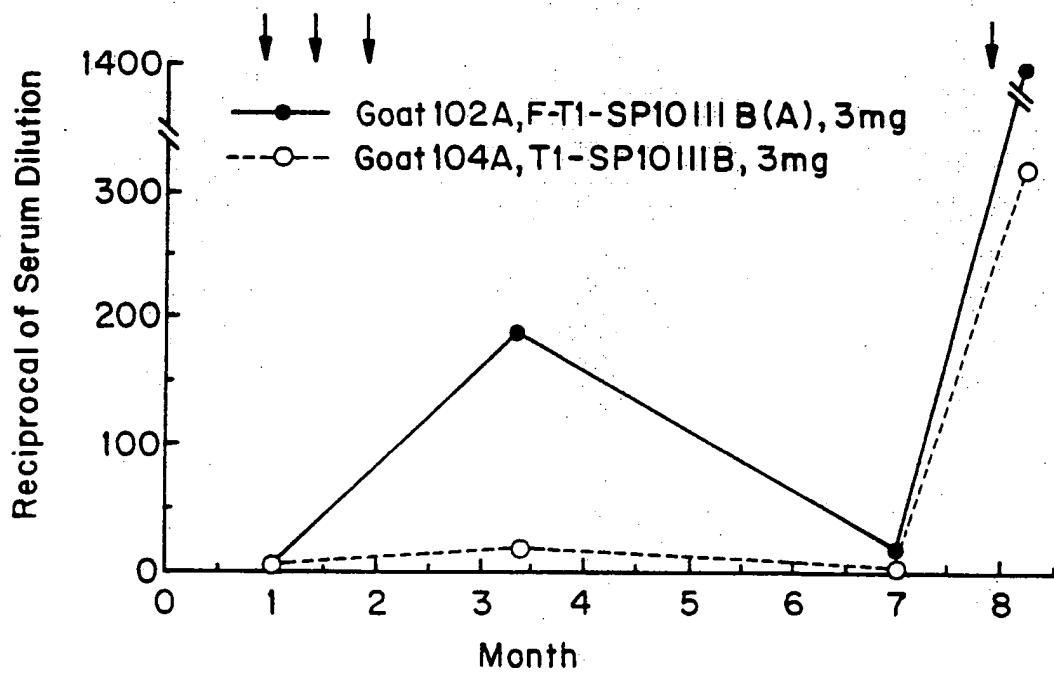
A.	CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/00, 37/02, 39/12; CO7K 5/00, 7/00, 15/00, 17/00
US CL :530/324, 327; 514/12, 14; 424/89
According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/324, 327; 514/12, 14; 424/89

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CA, BIOSIS, STN

C.	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, VOL. 88, ISSUED NOVEMBER 1991, HART ET AL, "PRIMING OF ANTI-HUMAN IMMUNODEFICIENCY VIRUS (HIV) CD8+ CYTOTOXIC T CELLS IN VIVO BY CARRIER-FREE HIV SYNTHETIC PEPTIDES", PAGES 9448-9452, SEE ENTIRE DOCUMENT.	1-15
Y	BIOCHEMISTRY JOURNAL, VOL. 269, ISSUED 1990, PAPADOULI ET AL., "ANTIGENIC ROLE OF SINGLE RESIDUES WITHIN THE MAIN IMMUNOGENIC REGION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR", PAGES 239-245, SEE ENTIRE DOCUMENT.	1-15

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 April 1993	04 MAY 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer AVIS DAVENPORT
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF EXPERIMANTAL MEDICINE, VOL. 167, ISSUED MARCH 1988, GOLDING ET AL., "IDENTIFICATION OF HOMOLOGOUS REGIONS IN HUMAN IMMUNODEFICIENCY VIRUS I gp41 AND HUMAN MNC CLASS II B 1 DOMAIN", PAGES 914-923, SEE ENTIRE DOCUMENT.	1-15
Y	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, VOL. 171, NO. 1, ISSUED 31 AUGUST 1990, MURAKAMI ET AL., "IDENTIFICATION OF IMMUNOGENIC REGIONS IN HUMAN THYROTROPIN RECEPTOR FOR IMMUNOGLOBULIN G OF PATIENTS WITH GRAVES DISEASE", PAGES 512-518, SEE ENTIRE DOCUMENT.	1-15